

Bill Magruder's Ship Comes Home

THOSE in Britain who proclaim that governments cannot adequately direct research should seriously ponder the way in which the United States Administration has for the third year tried—without much success so far—to make its expenditure on research and development more relevant—as the saying goes—to immediate needs. Two years ago, the budget of the National Science Foundation included a generous allocation of funds for the programme described as IRPOS—Interdisciplinary Research on Problems of Our Society. The disappointing discovery that suitable research proposals were alternatively unpointed or unglamorous led not to a retreat from the ambition to devise relevant research but to the National Science Foundation's programme called RANN—Research Applied to National Needs. But the foundation overplayed its hand at the budget hearing last year, and also blotted its copybook with Congress by seeming cavalierly indifferent to the needs of the universities and of research students, with the result that much of the increase of its budget was unspendable. Not seriously dismayed, however, the Administration has now gone on to propose a budget for the coming financial year which is likely—if Congress can be brought to heel in this election year—to see substantial federal spending on specific projects in applied research including rapid transport systems, sources of clean energy, protection from natural disasters and the like. The universities will not see much benefit from the increased spending, for the amounts which the National Science Foundation has at its disposal for research and development will increase only from \$409 million to \$455 million. The bulk of the increased expenditure will be administered by the government departments, although there may be some welcome fall-out from the new cancer programme. And the President has yet to say how much extra money will be made available to Mr William Magruder's office in the White House for projects of applied research.

The implications of the new federal budget for science and technology are still obscure, but there is at least a serious danger that the penury which the universities have endured for the past three years may now be crippling. Although the Administration estimates that the National Science Foundation will have an extra \$60 million to spend in the coming financial year, very little of this will find its way to the universities. There is still a long way to go to the goal of \$1,000 million a year that Mr William D. McElroy said at the beginning of his term as chairman would be his minimum demand. But as the years have passed, the universities have understandably become more than ever dependent on the National Science Foundation for the support of students and research. That is as it should be, for the old notion that there is bound to be a degree of safety in a multiplicity of grant-giving agencies of the federal government has long since been discredited by the shrinking of NASA and of the AEC. Although the Administration may be right in saying that research must somehow be made to

serve the community at large, its policies will come to nothing if the universities are unable to educate those without whom even applied research will not prosper.

The new budget is also shaky in its perpetuation of the space shuttle, sponsored by NASA. Gone are the days when the agency was rightly or wrongly committed to an objective—the landing of people on the Moon. Now, for practical purposes, it is committed merely to the construction of a machine, a device for carrying men to satellites in orbit about the Earth at a comparatively low cost. To safeguard this programme, plans have been dropped for sending rockets to distant planets, for completing the development of nuclear powered rockets and even for working out the ways in which the first stage of the shuttle vehicle could be returned to Earth and used again, originally the chief justification of this ambitious device. It must seriously be asked whether this assignment of priorities can be sensible. The men at NASA have consistently underestimated the benefits yet to be won from basic research within the solar system. It may always have been fanciful to expect that such things as Earth resources satellites could make a contribution to national and international life as important as telecommunications satellites, but it is mistaken to soft-pedal, as NASA is doing, the potential utility of knowing more about such things as the solar wind and even the nearby planets. By all appearances, for the sake of the shuttle, NASA is neglecting what it has become fashionable in Britain to call strategic research. If in Britain Rothschild has the edge on the Daintonites, there is good reason to think that Magruder needs a Dainton.

Cadmium off Avonmouth

THE flurry of excitement in the past week about the zinc smelting plant on the Avon is a splendid illustration of the kinds of issues into which environmentalists should sink their teeth. The plant is a technical innovation in the sense that the smelting process is carried out in a blast furnace, not by roasting the ore. In the two years since the plant has been in operation, it appears that the atmosphere within the factory has in some places been laden to an uncomfortable degree with lead. No doubt the operators, a subsidiary of the Rio Tinto Zinc corporation, are right when they claim that no workman has yet been damaged and that the limits set for zinc in the blood circulation have not been exceeded, yet it is bound to be a chancy business to make sure that no accidents will happen in circumstances in which the safety limits are as tightly drawn as they appear to be at Avonmouth. To be sure, it is a considerable benefit to all concerned that there are now laboratory techniques for telling where the threshold should be set. In the vulcanizing plants of the late nineteenth century, the only indices of risk were the overt symptoms of disease which the unfortunate workmen bore—and these were all too frequently regarded as



a part of the price of having a job at which to work. In present circumstances, there is no reason why laboratory techniques should not be used to define with some precision the conditions in which work can be safely carried out. One of the cheerful aspects of the operation of the Avonmouth plant is that the operating company and the medical advisers to the Trades Union Congress appear to regard the problem as a common concern. So, too, it should be. Occupational health is a particular if local part of the problem of the environment.

The zinc blast furnace appears, on the face of things, to have had other more regional effects. Two weeks ago, oceanographers from the University of Liverpool and the Marine Science Laboratory of the Natural Environment Research Council at Menai Bridge reported (*Nature*, **235**, 158; 1972) the distribution of copper, lead, cadmium and zinc in the coastal waters around Wales. One striking feature of the surveys which have been carried out is that there are comparatively large concentrations of heavy metals in the water of Cardigan Bay, presumably because of run-off from the heavily mineralized Cambrian Shield. Both the Mersey and the Upper Severn (into which the Avon is discharged) also, however, show up prominently, and it is only natural to infer that industrial enterprises of one kind or another are responsible. The particular anxiety which has caught the public imagination stems from the high concentration of cadmium off Avonmouth, where concentrations of 4.5 parts per thousand million have been recorded. Given that the open sea contains only about a fortieth of that cadmium concentration, it is plainly important to discover what the consequences may be for marine life in the Severn estuary. On the face of things, there is a case for controlling more rigidly the amounts of the metal discharged from Avonmouth. And even if, on present form, the total content of cadmium in the Severn estuary is unlikely to be more than a few hundred grams—comparable with the amounts in Cardigan Bay produced by natural run-off—it is plainly important to learn more about the movement of water in the Severn estuary so as to discover whether there is a risk that cadmium concentrations there are still increasing.

So is there yet another environmental poison to be stomached? That is what the taxpayers and sea bathers of Britain want to know. The first thing to be said is that the concentrations of cadmium so far detected are so small that they are unlikely to be directly a threat to human life. Whether, by concentration in marine organisms, they might become so is an open but exceedingly complicated question. Only if appropriate research is properly sponsored will it be possible to make reliable predictions. In the meantime, it will probably reassure many people to know that similar concentrations of cadmium and other heavy metals have been present in the Bristol Channel for more than a century (see *Nature*, **231**, 287; 1971), thanks—if that is the word—to the smelting enterprises which have flourished in South Wales. In other words, the time scale that governs the environmental consequences of cadmium in the Bristol Channel is longer than that which determines the health of those who work around the zinc blast furnace. This implies that there should be enough time for mounting a sensible programme of research in the Bristol Channel, but that the occupational health of those who work the blast furnace is more urgent. It is to be hoped that those who take this issue to heart will assign the priorities

correctly, however much less dramatic it may seem to battle for the cause of good working conditions.

Councils Speak Out

THE publication of the Rothschild report has at least had the virtue of persuading the research councils that they must break with the genteel conventions of the past and say what they think in public. This week, both the Medical Research Council and the Agricultural Research Council have published their comments on Lord Rothschild's proposals and, if the opinions are to be taken at face value, what they have to say is for practical purposes tantamount to a rejection of the framework of the Rothschild scheme even if neither of the councils goes so far as to deny that the customer-contractor principle has a place in the organization of applied research in Britain. Even though the government has promised to make its mind up quickly about the future pattern of organization, there is no doubt plenty of time and room for further manoeuvre and compromise. In the circumstances, it is quite possible that both the research councils will come to regret that they have not made a more constructive response to the Rothschild recipe for, whatever may yet be said on the subject, there is no doubt that the organization of scientific research in Britain will not emerge unchanged from the present re-examination.

The Medical Research Council is the more intransigent, and of course there is no doubt that its present organization will be more seriously affected than that of the Agricultural Research Council if ever the Rothschild recipe comes to pass. In its memorandum, the Medical Research Council says that in spite of the way in which research contracts with government departments have in recent years been the foundation of much of the research council's work, "the customer-contractor principle is inappropriate for most biomedical research". The argument is that there are few fields of research in which "further progress can be predicted with reasonable certainty". But this, of course, may be an argument that plays into Lord Rothschild's hands. Empiricists may sometimes be right to claim that it is good policy to concentrate expenditure on those fields in which some definite outcome can be expected and in which there is some hope that the benefits will exceed the cost. Although the Medical Research Council has done wonders in the past few years, as the article by Dr Max Perutz last week made eloquently clear (*Nature*, **235**, 191; 1972), it is by no means out of the question that the national well-being would be fostered by programmes of research in which laboratory and clinical research combined to yield some of the methods of treatment which recent developments have brought within sight. Among these the recent developments in the understanding of the relationship between cancer viruses and some forms of human cancer, breast cancer for example (see *Nature*, **235**, 74; 1972), is one rich field of opportunity. Does the Medical Research Council consider that present arrangements are adequate to mount a research programme large enough to offer a reasonable chance of success or even to make a decision for or against such an operation?

What riles the Medical Research Council most, however, is clearly the threat in Rothschild that roughly a quarter of its budget would in due course be transferred

to government departments "which implies removal of policy control from the council over a wide range of research". The council is right to complain that the £5.6 million that Lord Rothschild would sequester is a somewhat arbitrary figure, and that the council's continuing commitment to the staff of its own research establishments would imply that universities would be most seriously endangered if Rothschild came to pass. Yet one of the most interesting and in the long run potentially valuable implications of the Rothschild report is the implication that accountability in the sense of responsibility for research policy should rest with those who pay for it, government departments and the taxpayers whom they represent. Although the MRC may be right to complain that the Rothschild report would have been easier to follow if it had spelled out the ways in which the present performance of the research councils is deficient, Lord Rothschild in his original proposals made no bones about his wish that he who pays the piper should call the tune.

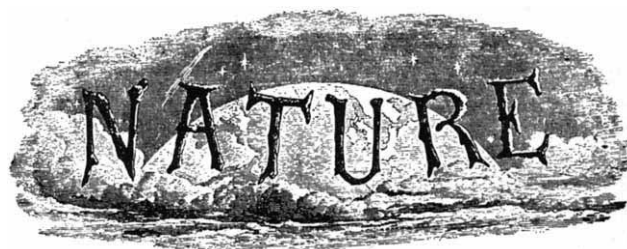
The Agricultural Research Council echoes this unwillingness to see a shift in the machinery for making decisions that would carry influence away from the research councils. The council says explicitly that it does not accept "the implication in Lord Rothschild's recommendations that the customer department alone is the best arbiter of the needs and priorities" for applied research. The council goes on to explain how widely based is its own machinery for gathering advice from "the real customers"—the agriculturalists—as well as from scientists and economists. The trouble is again a constitutional difficulty. Rothschild's challenge to the present system is a simple assertion that government departments have a responsibility to make decisions like these, and that it is a derogation of this responsibility for them to trust in the wisdom with which advice is gathered and policies formulated by a research council over which they have no direct control. It may be right and proper, in present circumstances, to complain that government departments are incapable of operating the customer-contractor principle sensibly, but if the government considers that its duty is to take a hand in the making of research policy, it is hard to see why it should be dissuaded from doing so on any but the most pragmatic grounds—that it is not yet competent to do so.

Both the research councils are sketchy in their account of the arrangements that they would like to see for the future planning of research and development. The MRC advocates a redefinition of responsibilities between itself and the health departments, which "should be distinct but complementary", a review of resources and of the present pattern of expenditure and a system of association between the staffs and committees of the council and the departments so as to make for closer collaboration. The Agricultural Research Council advocates closer collaboration but says that it would wish to restrict the proportion of commissioned research in its portfolio to less than 20 per cent. In reality, however, these proposals are unlikely to meet the government's present need. In his evidence to the Select Committee on Science and Technology last week, Lord Rothschild was explicit in his declaration that the "determination of priorities" should be "the responsibility of the public, represented by Parliament". No amount of juggling with committees will weaken this principle for violating which, Lord Rothschild pointed out, "the great Sir Francis Bacon was impeached". This is why the best defence of the re-

search councils and their autonomy—their monopolistic position as Lord Rothschild called it—should best be an acceptance of the customer-contractor principle wherever the government departments can demonstrate that they are competent to exploit it.

This is why it is unfortunate that Lord Rothschild came out last week against a trial of his proposals in some narrow field. He said that such an experiment "would not be useful, since the results of any experiment would be interpreted differently by protagonists on either side". But is that really a serious danger? After all, the government will have the final say, but if it could demonstrate in a reasonable period of time, the next two years perhaps, that some part of the budget of one of the research councils, the ARC for example, could be dealt with intelligently by the Ministry of Agriculture, Fisheries and Food, it would have gone a long way to disarm the hostility of those who fear that the Rothschild report is a recipe for less good research. In the process, it would no doubt find itself providing a demonstration that if commissioned research is more effective, it is also likely to be more expensive, as well as a test of the nerve of the civil servants in the departments other than defence departments at seeing costs rise to exceed the first estimates. After all, programmes of applied research in the civil field are potentially as hard to predict in advance as those for the development of new types of aircraft. In the circumstances, it will be a great misfortune if the government looks for too rapid an implementation of the principle to which it has committed itself.

100 Years Ago



A CLEVER application of science to commercial purposes has been made by an Italian gentleman, M. Eugenio de Zuccato, of Padua. By means of the invention any number of copies of a manuscript or design, traced upon a varnished metal plate, may be produced in an ordinary copying press. The *modus operandi* is very simple. To the bed and upper plate of a press are attached wires leading from a small battery, so that when the top of the instrument is screwed down the two metal surfaces come into contact, and an electric current passes. An iron plate resting upon the bed of the press is coated with varnish, and upon this surface is written with a steel point any communication it is desired to copy. The letters having thus been formed in bare metal, a few sheets of copying paper are impregnated with an acid solution of prussiate of potash, and placed upon the scratched plate, which is then subjected to pressure in the copying press. An electric current passes wherever the metal has been left bare (where the writing is therefore), and the prussiate solution acting upon the iron, there is found prussiate of iron, or Prussian blue characters, corresponding to those scratched upon the plate. The number of copies that may be produced by this electro-chemical action is almost unlimited, and the formation of the Prussian blue lines is, of course, instantaneous. The patent, which is, we believe, the property of Messrs. Waterlow and Sons, forms a remarkable instance of science serving as handmaiden to the man of business.

From *Nature*, 5, 292, February 8, 1872.

OLD WORLD

Lord Rothschild in the Dock

LORD ROTHSCHILD successfully parried the thrusts of the Select Committee on Science and Technology at its first public meeting held last week to enquire into the British government's policy on research and development. In a public defence of his report—published in the green paper *A Framework for Government Research and Development*—Lord Rothschild claimed that he had been misinterpreted in the press and that his position as a government employee had prevented him, until now, from refuting his critics.

Before his encounter with the politicians, Lord Rothschild circulated a memorandum that set out what he thought were the popular misconceptions held by his opponents. In the memorandum he dismisses the arguments that Table 4 of the report is inconsistent with his definition of applied research and development. He says that the definition set out in paragraphs 6 and 7 was never intended to be treated as narrowly as it has been. The definition of applied research as that "with a practical application as its objective" was meant to describe the nature of the work rather than its purpose.

Lord Rothschild also regretted the "emotive effect" of the use of the phrase "customer-contractor" with all its connotations such as formal documents and witnesses. He stressed that the word contract could equally well be replaced by commission and customer by user. Lord Rothschild reaffirmed his belief that applied research and development should only be done if there was a customer who knew what he wanted and was prepared to pay for it. He qualified this by saying that in his view "judgment about whether such research should or should not be done cannot be exercised entirely by those who will be engaged on the research".

The level of research and development carried out by the government would probably go up if his recommendations were implemented in full, said Lord Rothschild. This belief, he emphasized, was based on a hunch rather than on any facts, but he did stress that, by contrast with what several people were saying, his report did not imply a reduction in the level of basic or strategic research.

Another fear of some scientists was allayed when Lord Rothschild said that he was "in no sense recommending a creeping takeover by the government of direct control of all research done by the research councils". He said that he regarded the research

councils as monopolistic because they are not scientifically accountable for their programmes. He illustrated this by quoting from the Dainton report the amounts spent by the MRC during 1968–69 on research into cardiovascular disease (£254,000), ageing (£14,000), molecular biology (£867,000), and dentistry (£57,000). Lord Rothschild did not criticize the priorities implied by the figures but he wanted to know who was in a position to query them.

Mr Airey Neave, the chairman of the select committee, asked Lord Rothschild why there was a general impression that his report dealt chiefly with the research councils, whereas in fact only one chapter was concerned with their future. Lord Rothschild confessed that he did not know how this fallacious view had arisen. He followed this up by asking Mr Neave why the select committee had sought evidence almost exclusively from the research councils and had not included chief civil servants of the various departments. Mr Neave said that "you can leave it to us to decide whom we want to see". Mr Neave added that the list was not necessarily complete and that both Mrs Margaret Thatcher and Lord Jellicoe would give evidence at a later date.

The select committee expressed its concern that Lord Rothschild considered the customer-contractor principle to be self-evident. In reply, he said that his thesis was that in a democratic society bodies with a substantial share of the nation's resources could not be allowed a position of autonomy. He was challenged by the committee on the grounds that a self-evident proposition was unscientific, but Lord Rothschild countered by saying that he would use the term in a scientific context.

Lord Rothschild commented on the fact that there had been a great deal of discussion to the effect that the objectives of applied research and development could not be specified as precisely in the biological sciences as they could in the physical sciences. He said that this did not mean that the customer-contractor principle should not be applied in the biological sciences and that an effort should be made to specify the objectives precisely. Thus, far from agreeing that the principle could not be applied in medical, ecological and agricultural research, Lord Rothschild said that a lack of knowledge is "no excuse for not trying and for relegating as much research as possible in these fields into the 'pure' and 'strategic' categories, convenient

as this may be for the scientists in contradistinction to the nation".

Lord Rothschild said that the final decision on what percentage of the MRC, ARC and NERC budgets to transfer to the appropriate departments would be made by the government, but that a trial period, as demanded by some scientists, would not be practical. According to Lord Rothschild, "a trial of my proposals on an experimental basis would not be useful, since the results of any experiment would be interpreted differently by protagonists on either side of the argument".

Lord Rothschild did not think that the research councils would protect their position by moving further into basic research if his proposals were implemented, and he said that "this would imply a degree of irresponsibility which I do not believe exists". And anyway, as Lord Rothschild pointed out, the government holds the whip hand and the science budget could be reduced as a counter to any such moves by the research councils.

The report took six months from conception to delivery to the cabinet, and one of the concerns of the select committee was that this time was insufficient for a considered judgment to be arrived at. The report was prepared chiefly by Lord Rothschild with the assistance of one member of the Central Policy Review Staff and with some help from the staff of Sir Alan Cottrell's department. During this time discussions were held with the heads and staff of research establishments other than those belonging to the research councils, the head of the Confederation of British Industry, and the Trades Union Congress.

Lord Rothschild stated that there was a "marked weakness in the scientific staffs of departments". In particular, he considered the scientific staff of the Department of Health and Social Services to be inadequate. Further, the Ministry of Agriculture, Fisheries and Food did not, in fact, have a chief scientist. He did, however, feel that the first priority was to establish an efficient customer-contractor relationship and that a chief scientist should then be appointed.

During the hearing Lord Rothschild affirmed that he still stood by the content of the report, but that he did regret not having made it longer. He felt that a great deal of the misinterpretation had arisen because he had not been too specific, and that a few extra paragraphs to qualify his point of view would have been worthwhile.

EDUCATION

Along with Lord James

It comes as no surprise that a report on the training of science and mathematics teachers published this week recommends continuing training for teachers during their careers. The Royal Society, which published the report, is, however, at pains to point out that this is not an echo of the James report (see *Nature*, **235**, 186; 1972) as it was approved for publication before the long awaited appearance of the recommendations of the inquiry of Lord James and his colleagues. Nevertheless this document is bound to be an anticlimax (*The Training of Teachers of Science and Mathematics*, the Royal Society, 1972).

The report recommends that in the next six years all teachers of science and mathematics should spend at least one week a year undergoing in-service training. The report also recommends that any teacher should be able to extend his knowledge of science, educational theory or curriculum development by attendance at short or long courses so that he may increase his professional competence. This the report calls "post-certificate study" as it is felt that the use of the term "in-service training" implies that the teacher does not know his job and so must be avoided.

The present report goes further than the James report in that it recommends three stages of post-certificate study. The first stage would be for recently qualified teachers and the courses would be designed to improve techniques and overcome any deficiencies in subject knowledge. The second stage courses would be for teachers with several years' experience and would be designed to improve and update their knowledge. The final stage would be for teachers who were aiming at posts that carried substantial responsibility. The Royal Society working party did not feel that salary increments should be awarded to teachers merely for attending a course but that the incentive for attending would be increased opportunities of promotion.

A recommendation that is bound to meet with general approval is that science, mathematics and technology should form a part of the education of all children. The working party feels that it is important that children appreciate how scientific information is obtained and also how this information is described in simple mathematical terms. A stumbling block to this end, as admitted in the report, is the scarcity of qualified teachers. The working party deplores the teaching of factual knowledge—which it says is easily forgotten—and it welcomes the Nuffield approach to science teaching in schools. It also feels that it is essen-

tial for both biology and physical sciences to be taught and that, in particular, no biology should be taught without the pupil having a background of physical sciences.

The working party, under the chairmanship of Professor Sir Neville Mott, feels that a greater encouragement should be given to students who possess higher degrees to enter the teaching profession. The report states that a PhD needs "an introduction to the techniques of teaching and to new curricula". While not coming out openly and recommending that a PhD receive a full salary while he is being trained, the committee nevertheless says that there is a strong case for this to be the normal procedure. It also feels that for the PhD there should possibly

be an alternative to the full one year course of professional training. The committee was undecided what the situation of an MSc graduate should be, and thought possibly he should be treated like a first degree graduate.

The working party also considered that scientists who have spent some time in industry should be encouraged to enter the teaching profession and it recommends that special courses be set up to train these people. It recognizes that it would be difficult for these people to take a full year's course of retraining although this would be desirable. It therefore declares that there is a case for having part time courses—even if these prove costly. It feels that married women would also benefit from such part-time courses.

SCIENTIFIC EQUIPMENT

Assessing Sophistication

THE increasing sophistication of scientific equipment used in universities caused price increases in real terms of about 9.5 per cent a year between 1956–57 and 1967–68. This is one of the findings of an investigation of equipment costs in university science and engineering departments carried out under the auspices of the Council for Scientific Policy (*Science Policy Studies No. 5*, HMSO, 1972; £0.325). The source of the information used in this report was a questionnaire returned by sixty-nine departments at fourteen British universities.

For the purposes of comparison, departments were divided into four categories—biological sciences, physics, chemistry and geology, and engineering and metallurgy. One of the problems in any survey of this type is to decide to what extent personnel in a university science department (academic staff, research students and undergraduates) should be included in the calculations of *per capita* expenditure on equipment. There are several ways of defining the "unit scientist" and the authors of the CSP report consider the inclusion of all personnel in the calculation, or all researchers, or all researchers and a proportion of those not engaged in research activities. Similarly, equipment costs have to be divided into recurrent costs

(footed, for example, by the University Grants Committee and the research councils) and those non-recurrent grants which the UGC makes to departments moving into new buildings or extensively refurbishing old ones.

The mean annual growth rate of all equipment expenditure per unit scientist does not seem to depend much on how the unit scientist is defined, but the growth rates for recurrent equipment costs are lower when only researchers are included. The report attributes this difference to the fact that the number of researchers is increasing more rapidly than the total personnel. The greatest annual rate of increase of *per capita* total equipment expenditure was in the biological sciences (see Table), but all the figures for recurrent costs are remarkably similar.

In assessing the contribution of the so-called sophistication factor to equipment price increases, the report considers only recurrent costs and makes an allowance of 0.7 per cent for the greater relative growth of the number of researchers. The sophistication factor turns out to range between 9.3 per cent for biological sciences and 10.4 per cent for engineering and metallurgy; for all sciences the figure is 9.5 per cent. The report does, however, point out that although the concept of sophistication becomes distorted when expenditure is curbed, the twelve years covered by the survey was a period of relatively generous funding for science.

Mean Total Equipment Expenditure (*per capita*, all personnel) and Annual Rates of Increase (1956–68)

	Total <i>per capita</i> expenditure (current prices) £	Annual increase (total) %	Annual increase (recurrent) %
Biological sciences	70	19.7	10.1
Physics	176	9.3	9.8
Chemistry and geology	105	15.0	11.0
Engineering and metallurgy	155	9.7	11.2
All sciences	127	12.3	10.3

SOVIET SCIENCE

Rationalizing Research

from our Soviet Correspondent

THE Soviet plan for establishing science centres around new and existing universities and institutes continues. The latest in the series, the West Ukrainian and Uralian centres, are interesting in that they reveal a double aim in the plans for these centres, being concerned with the study of immediate and local problems and also with basic research on the all-Union scale.

West Ukraine is an area which did not become part of the Soviet Union until 1939 and so academic traditions of such centres as L'vov have always tended to be western-looking rather than Russian-orientated. For this reason, perhaps, the new centre, to be based on fifteen institutes, museums, libraries and similar establishments in L'vov, Uzhgorod, Chernovtsy and Ivan-Frankovsk, is seen to be important in the promotion of joint research between the Soviet Union and her neighbours of the socialist bloc, and will even have a special department at Uzhgorod, devoted to the economic history of the eastern bloc.

In the pure sciences, however, there seems little direct connexion between the area and the centre it houses. There is to be a considerable emphasis on physics. In Uzhgorod, the Institute of Theoretical Physics of the Ukrainian Academy of Sciences has recently opened a department of hadron theory, which is working in close conjunction with Kiev, Dubna, Serpukhov and the Lebedev Institute in Moscow. In L'vov, the Institute of Physics and Mechanics is to become a particularly important part of the new centre, specializing in problems of measurement, information processing, new methods of prospecting — including aerial reconnaissance of magnetic fields, thermal regimes in the post-weld stage of manufacturing machine parts and problems of the lifetime of machines — all of which are of all-Union concern rather than being a purely Ukrainian matter.

In the Urals, however, the picture is somewhat different. The Uralian science centre, with bases in the Sverdlovsk, Perm and Tyumen provinces, which has been operating for almost a year and already employs some two thousand scientific workers of all grades (including four full Academicians) seems to be concerned much more with local scientific problems. Here, too, there is an Institute of Mechanics and Mathematics, specializing in control processes, and is already known for its work in mathematical physics.

The principal task of the Institute of Geology and Geochemistry will be to

study the mineral deposits of the Urals, and the structure of the Earth's crust and upper mantle in the region. An Institute of Flora and Fauna Ecology will study the biological resources of the area. The Institute of Economics will be concerned with planning and development in the area, including forecasting of the productivity of the area up to the year 2000, and will also study problems of demography and the influx of labour into the area.

COMMUNICATIONS

Through a Storm Clearly

As the demand for telecommunications increases, the need to open up new regions of the electromagnetic spectrum becomes increasingly important. Mr W. J. Bray, Director of the Post Office Research Department, pointed out last week that it will only require 1 per cent of existing telephone customers to switch to viewphones to double the present telecommunications requirements in Britain. Looking ahead to the late 1970s and early 1980s when viewphones should be available, the Post Office and the Science Research Council are now working on opening up the 10 to 100 GHz range of the radio spectrum. The existing microwave network in Britain operates at 2, 4 and 6 GHz; above 10 GHz serious problems of fading and multi-path transmission are encountered because of atmospheric disturbances.

It is the problem of attenuation caused by rainfall which is now being tackled with a network of experimental transmitters set up in East Anglia, between the research station at Martlesham and the radio station at Mendlesham. The first important feature to emerge from the studies undertaken is that rainfall of the intensity needed to cause serious attenuation (50 to 100 mm h⁻¹) occurs only in severe storms, over a core less than 1 km in width.

These intensities of rainfall are rare, but the attenuation due to them must be overcome. There are two ways to solve the problem. One is literally to get round the storm, by having a system of microwave links with at least two paths between each station and incorporating automatic switching so that the best signal is always taken by the receiver. Alternatively, the repeater stations between transmitter and receiver can be close enough to rule out significant fading even in the heaviest rain. The exact spacing necessary for these repeater stations will clearly be important in determining which system is finally chosen by the Post Office.

But it seems certain that in one form or another telecommunications must move into the 10 to 100 GHz range in order to meet future needs.

SCIENCE POLICY

Rothschild and Industry

THE current controversy over the Rothschild and Dainton reports on the reorganization of research and development has generated more heat than light according to Lord Sherfield, Chairman of Industrial and Commercial Finance Corporation Ltd.

Lord Sherfield pointed out that the massive re-examination of government spending on research and development — witness the Rayner, Rothschild, Dainton and Docksey reports (the Docksey findings have yet to be released) — has been undertaken not simply for the sake of change but because real worries exist. Spending *per capita* on research and development in Britain is higher than in West Germany and Japan but the return is low. This, Lord Sherfield suggested, is due to a failure of marketing and management.

While appreciating that costly projects will in future have to be undertaken on an international level, Lord Sherfield said that as far as possible the management of them should be kept within one nation. Experience has also shown that where cooperation is essential then the more basic the programme the better — the record of Cern is better, for example, than that of the European Launcher Development Organization (Elido).

Dr Walter Marshall, Director of the Research Group of the UKAEA at Harwell, said that he approved of Lord Rothschild's general philosophy and of the emphasis Rothschild placed on multi-functional laboratories. Harwell, he said, is just such a laboratory, and carries with it the psychological advantage that in such a laboratory, with a large effort put into applied research, it is much easier to decide to phase out good programmes in favour of even better ones.

But when it came to industry's views on the relationship between government, industry and research and development, it was apparent that it felt it had bigger fish to fry than the research councils. Mr Handel Davies, Technical Director of BAC, pointed out that current arguments over research council spending were concerned with less than 5 per cent of national expenditure on research and development. The amount spent in industry is much more — £500 million a year — of which the government spends about two fifths. But the speakers from industry wanted more consultation with the government — before during and after the completion of research programmes. It was pointed out that there should be more awareness on the part of industry, the research councils and the National Research Development Corporation of each other's problems.

NEW WORLD

Echoes of Rothschild in Canada

by our Washington Correspondent

IF intensive study and debate is a guarantee of success in science policy making, then Canada should have the most efficient and productive scientific effort in the Western world. Almost monthly, the Canadian science policy machinery cranks out a report examining in detail some aspect of the country's investment in science and technology, but it is perhaps a measure of the difficulty of first establishing a consensus, and then of putting policy into action, that each report seems to be more critical than the one which preceded it.

The latest in this long line, a weighty document prepared by a committee of the Senate under the chairmanship of Mr Maurice Lamontagne, is no exception*. It is a call for radical reform, both in the level and direction of investment and management of Canadian science, although the far reaching nature of the committee's recommendations tends at times to get lost in the ponderous and highly detailed discussion with which they are launched.

The problems to which the Canadian Senate committee addresses itself are common enough: the need to stimulate investment in industrial research, the desire to regulate investment in basic research to ensure maximum returns, in terms both of scientific advancement and economic gain, and imprinted on the whole discussion is the problem of ensuring that the number of qualified scientists and engineers roughly matches the number of jobs available. But the solutions are as elusive as the problems are complicated, and the Senate committee's recommendations will inevitably be unpalatable in some quarters.

In some respects, the problems for Canada are increased because of its proximity to the United States, and especially because of the influence on the Canadian economy of large United States corporations. But, like any country with a relatively small economy, Canada faces many painful choices in deciding on which areas and at what level research should be supported, and, like its partners in the Western world, the Canadian government is increasingly looking for ways to increase investment in utilitarian research which is likely to lead to benefits for the country's population.

With those constraints in mind, the

*A *Science Policy for Canada, Volume 2, Targets and Strategies for the Seventies*, prepared by the Senate Special Committee on Science Policy.

Senate committee attempts to set out a list of targets for Canadian science policy in the 1970s, and discusses recommendations for achieving them. Overall, the committee believes that expenditure on science and technology should grow at an average rate of 15 per cent a year, to reach 2.5 per cent of the Gross National Product by 1980 and the magic 3 per cent by 1985. But within this global sum, there should be considerable shifts in emphasis from the present relatively high preoccupation with basic research, to a more concerted effort in applied research and development.

In detail, what the committee suggests is that the proportion of the total scientific effort devoted to basic research should shrink to about 10 per cent by 1980, and that the agencies which hold the government's purse strings for basic research should be more selective in the way they dole out their money. This recommendation, the committee acknowledges, would represent a major shift in Canada's research and development effort because in 1967, for example, the country was spending 23.1 per cent of its scientific outlays on basic research. But, before too many hands are raised in horror at the prospect of cutbacks in the universities, the committee quickly points out that the target of 10 per cent still means a doubling of expenditure on basic research between now and 1980.

In justification of this target figure, the committee points out that the division of research funds in Canada between basic and mission-oriented research and development is out of line with most other industrialized countries, and that in spite of this emphasis, "while it may be said that in the course of the last decades (Canada has) established a solid foundation in basic science . . . the overall contribution of Canadian-born scientists residing in Canada has not been outstanding". Nevertheless, a long discussion of the merits of basic

research, not the least being that Canada needs its own pool of good scientists to ensure efficient importation of research results from outside her borders, convinces the committee of the necessity of strengthening fundamental research efforts.

But, while the universities emerge slightly bloodied from the suggestion that they should receive a rather smaller proportion of the total expenditure on research and development, the machinery by which grants are distributed comes in for much greater punishment. The committee calls, in fact, for a totally new administrative organization to replace the present National Research Council, which parcels out grants to universities and also manages its own laboratories, the Medical Research Council, which as its name implies trades in grants for health research, and the Canada Council which supports the arts and humanities. The suggestion is that three new research foundations, catering for the physical sciences, the life sciences and the social sciences respectively, should be established, and that their work should be coordinated by a committee to be called the Canadian Research Board.

The rationale behind this proposal can be heard echoing around most corridors of science policy offices—the Medical Research Council, the committee says, has become too narrowly involved in medical research, while other life sciences are the responsibility of the National Research Council, and without a strong body directing overall operations of the proposed research foundations, the relative funding levels for different areas of research could not easily be apportioned. This proposal thus naturally leads to the second chief item which is likely to raise some notes of dissension in a few areas of the academic community: the committee suggests that priority should be given

Table 1 Estimated Total Expenditures on R & D in Canada, 1969

Source of funds	Sector of performance			Total, sources of funds
	Business enterprise	General government	Higher education and private non-profit	
		(millions of dollars)	(millions of dollars)	
Business enterprise	312	3	1	316
General government	56	359	241	656
Private non-profit	—	—	9	9
Higher education	—	—	52	52
Foreign	19	3	2	24
Total, performers	387	365	305	1,057

first to the social sciences and humanities and second to the life sciences.

Described as "a drastic change in priorities", this shift of emphasis would take place because the social sciences and the life sciences have consistently been singled out as poor cousins in many of the reports on basic science in Canada, while the physical sciences have carried off the bulk of funding for basic research. And, as an extra sting in the tail for the physical sciences, the committee suggests that Canada should keep out of big science projects funded on a national basis, and that grants should go chiefly to projects that employ only one or two researchers in a single discipline. (Although the committee adds that if interdisciplinary research projects look like proving successful, they should be supported.)

The proposed foundations would concern themselves only with giving grants for basic, curiosity-oriented research, and the committee suggests that the actual operation of government research establishments should be turned over to another new body—a national research academy, in which would be concentrated all the research activities of the government. Moreover, echoing the Rothschild report, the committee suggests that a substantial proportion of the work of the proposed academy should be performed for government and private industry on a contract basis.

The committee is, however, chiefly concerned with the relatively low level of investment in research and development in Canadian manufacturing industry, an environment which it calls "unconducive to industrial innovations". To help stimulate industrial research and development, the committee puts forward a multi-pronged line of attack, which aims to transfer some of the government's applied research activities to private industry and to the universities, and to make available the risk capital necessary to launch technological innovations.

In short, what the committee proposes is that the Minister of State for Science and Technology should take stock of all the research and development programmes of government departments, to see which should be dropped or contracted out to universities and industries. In the longer term, all grants and other devices aimed at stimulating research and development in industry should be integrated into one programme administered by the Department of Industry, Trade and Commerce. Industrial organizations should themselves set up task forces to look at ways to improve the efficiency, innovative capacity and international competitiveness of individual firms through mergers and so on. The aim of the enterprise would be to create a better environment for innovation and to shift more of the burden for

financing research and development from the shoulders of the government to industry.

As for manpower, Canada is at present suffering similar experiences to her North American neighbour, and the prospect seems to be that the output of qualified scientists and technologists from the universities will greatly exceed the supply of jobs in the years ahead. But imprinted on the overall unemployment situation is what the Senate committee describes as "some curiously convoluted details". For one thing, many industrialists claim that they must look abroad for a substantial proportion of their personnel for industrial research, and that "while an abnormal number of fellows in the physical sciences come from abroad to take advantage of financial assistance in Canada, a high proportion of young Canadian social scientists use their scholarships to go abroad". The whole situation, the committee believes, represents "a serious misallocation of national funds", and it suggests that the Minister of State for Science and Technology should conduct a thorough reappraisal of the government's scholarship and fellowship schemes, taking into account the likely effect of the Senate committee's own proposals on the manpower situation.

In short, the report calls for closer integration of the government's activities in research and development, and an overall shift of emphasis towards industrial research and development, with private industry taking a greater share of the funding. In some respects, the Canadian government has already gone

a short way towards meeting these objectives by setting up a new ministry concerned with science and technology; the new minister, Mr Alistair Gillespie, an economist, was appointed in August last year. One thing Mr Gillespie will not be short of is advice—the Senate committee is aiming to produce two more volumes of its report, and the Science Council of Canada also reports to him. The heated discussion which this report will doubtless spark off in the universities could be as much of a help in defining objectives for the 1970s as the report itself.

ASTRONOMY

VLA Welcomed

by our Washington Correspondent

REACTION among astronomers to the Administration's decision to give the green light to the Very Large Array system of antennas has generally been one of approval. But it has also been tinged with regret that the decision was not made five years ago when the proposal was first seriously put forward. Nevertheless, the decision to proceed with construction in the 1973 financial year, if Congress approves, should give radio astronomy a much needed boost.

The Administration's approval came in the form of a \$3 million request in the budget of the National Science Foundation, which will be the first instalment of about \$63 million for the instrument. It is expected to take between five and ten years to build, depending on how quickly the funds are made available, and although no definite site has yet been designated the south-western part of the United States will be the most likely area. The siting is constrained by the need for a flat area of 35 km as far south and as high as possible to eliminate atmospheric disturbances.

The instrument will consist essentially of 27 antennas mounted on tracks in a Y-shaped formation, and it is described by the National Radio Astronomy Observatory (which will take charge of the instrument) as the radio version of the 200-inch telescope—a high-resolution image forming instrument capable of producing pictures of radio sources comparable with the finest optical telescopes.

The VLA will be used to study the physics of extragalactic radio sources, stellar structure and evolution and eventually also for interstellar spectroscopy. Initially, it will operate with a resolution of 1 second of arc at 2,695 MHz and 0.35 seconds of arc at 8,085 MHz, producing more than 10,000 picture elements in less than 12 hours. When interstellar spectroscopy is added, the instrument will also operate at another wavelength, but firm decisions have not yet been made.

AMERICAN PHYSICAL SOCIETY

Panofsky Elected

DR WOLFGANG K. H. PANOFSKY, Professor of Physics at the Stanford Linear Accelerator Center, was voted vice president elect of the American Physical Society at the society's annual meeting last week. This means that Panofsky automatically becomes vice president next year and president in 1974. Director of SLAC since 1961, Panofsky is an active advocate of arms control. He is a consultant to the Arms Control and Disarmament Agency, and a vocal opponent of the Safeguard anti-ballistic missile system. Among his scientific credentials are membership of the National Academy of Sciences, recipient of the E. O. Lawrence Memorial Award and the National Medal of Science. Philip M. Morse of MIT took office as President of the APS for 1972 at the same meeting.

How Green was my Paper

Sir Gordon Sutherland, Master of Emmanuel College, Cambridge, offers his views on the government green paper *A Framework for Government Research and Development*.

THE government's green paper is entitled "A Framework for Government Research and Development". It is just over one page in length; it never refers to a framework but only to a customer-contractor principle, which it unequivocally endorses for applied research and development; it expresses the belief that, subject to this principle, it would be right to preserve the research councils under the sponsorship of the Department of Education and Science. The paper is consequently rather a pale green, because the commitment to the customer-contractor principle seems absolute enough to make that vital section as white as any white paper. From the title one is led to expect proposals for changes in the present organization comparable, for instance, with those proposed in the Trend report of 1963. The brief government paper makes no mention of organizational changes; the Rothschild report (the first appendix) leaves the present framework unaltered, while the Dainton report (the second appendix) merely proposes a Board of the Research Councils as a substitute for the Council for Scientific Policy.

One wonders what has happened to the Central Advisory Council for Science and Technology (CACST) which was established in January 1967 "to advise the government on the most effective strategy for the use and development of our scientific and technological resources". So far as I am aware, this prestigious body in five years has only published one small report—in 1968—"Technological Innovation in Britain". If this top body has been disbanded, why doesn't the government say so and explain who is now giving it advice on "the most effective strategy"? Presumably it is Lord Rothschild's Central Policy Review Staff, because his report states (paragraph 58) that any central body exercising general oversight of government and even national research and development would be superfluous since, "as far as pure or fundamental research is concerned, this oversight is exercised by the research councils (especially the SRC) and the Council for Scientific Policy", while for applied research and development "the

inescapable conclusion from this report is that general oversight would serve no useful purpose and indeed would negate the principles put forward in the report".

This flat assertion is considerably weakened by the realization in the following paragraph that some co-ordination at least of applied research and development may be required. This job is summarily assigned to the Chief Scientific Adviser, who has also been given the unenviable task of monitoring the implementation of 54 of the 55 recommendations of the Rothschild report. He is recommended to set up *ad hoc* committees to deal with such problems "rather than set up yet another scientific advisory organization, unless events in the future make it desirable to reconsider this question". I suspect that future events will lead to such a reconsideration.

So there is no new framework; only part of what we assumed to be the existing one has been quietly removed and nothing has been put in its place. It is true that the CACST never published a strategic plan. Maybe it never had one but one can't help feeling that if Lord Rothschild had shown such a body a draft of his report they might have been able to prevent him antagonizing so many of the scientists and administrators on whose cooperation and goodwill the success of his proposals must ultimately depend.

I have a great deal of sympathy with what appears to me to be one of Lord Rothschild's main objectives, that is to make each government department fully responsible for the research and development which is necessary for it to achieve its contribution to the national welfare. For several years, I have been advocating that we abandon a doctrinaire devotion to the over-hallowed Haldane principle and take a look at the US framework in which each department is responsible for the scientific research, basic as well as applied, which is required to fulfil its missions. The luxuriant flowering of basic research in America in the post-war years was largely due to the philosophy pioneered by Alan Waterman at the Office of Naval Research that basic research

related to the mission-oriented research must be generously supported in universities and also in government laboratories.

It is interesting that over 20 years ago the old Advisory Council on Scientific Policy in its first annual report (Cmd 7465 of July 1948) stressed the need for the executive departments to "assume a more positive role than hitherto on the organization and direction of research required for their own purposes", and concluded that "they would have to employ scientific staffs corresponding approximately to the operational research units attached to the service ministries during the war".

The Rothschild report attempts to spell out in detail how this might be achieved, not by setting up organizations in each department additional to the research councils but by using the research council framework, at least for medical, agricultural and environmental research. It proposes that the chief executive officer of each of these research councils should be the controller of research and development in respect of applied research and development. Moreover, the choice of these executive officers and their part-time chairmen must be approved in each case by the appropriate departmental secretary of state. Administratively, this seems a very strange arrangement. The chief executive officer is to use the customer-contractor principle for the applied research and development which the chief scientist of his department tells him is required and which is therefore paid for by his department; but for the related basic research the chief executive officer is responsible to the Department of Education and Science which provides the remainder of his budget. Can one man serve two ministers or a research council satisfy two ministries? The Rothschild scheme will tend to perpetuate and aggravate the separation between applied and basic research in departmental thinking. Instead of adopting the principle of departments sponsoring basic and strategic as well as tactical and applied research, the departments will feel that it is the function of the DES to look after all basic and most strategic research. In other words the Haldane principle is still being observed but in a modified form which (judging by the correspondence in the press) is clearly not acceptable to a great many scientists.

The Dainton report is the complete antithesis of the Rothschild report in style as well as in content. Whereas Roths-

child makes 55 specific recommendations (many on matters of detail) as a result of a number of assertions with virtually no discursive argument, Dainton puts forward essentially one recommendation based on a new classification of the types of research, several pages of history and much general discussion. His report carefully avoids being specific, apart from the composition and functions of the proposed Board of the Research Councils, which it recommends should replace the present Council for Scientific Policy. I do not find the arguments for the change very convincing. While it is probably true that the present CSP is to a considerable extent a board of the research councils, the concept that the top body in science policy in Britain should be a body which coordinates the work of a number of "autonomous" research councils is very surprising. The Dainton report also preserves a discreet silence about the Central Advisory Council for Science and Technology, which was set up when I was a member of CSP, precisely because the CSP was unable (by its constitution) to take an overall view of government science policy and expenditure. The new board would be given powers to allocate expenditure to the individual research councils, to create and abolish research councils, all of which would be financed by the DES. It would know what research is required by other departments by having members from these departments. However, "so as not to make the board too big", the maximum number of other departments represented would be three and it is even suggested that these three "should be appointed perhaps in rotation". That would be really ludicrous.

The following seem to me to be the desiderata which should be satisfied by any framework for government research and development.

- Each department of government must make full use of science and technology in trying to achieve its objectives.

- Where the necessary science or technology does not already exist, the department must ensure that the required research and development are carried out satisfactorily and must have the financial and other means to enable it to do so.

- It is the responsibility of the Cabinet to set up an organization for government research and development by which these two objectives will be fulfilled as efficiently and economically as possible.

In the original Haldane proposals the first requirement was to be satisfied by the appropriate minister having "at his disposal and under his control an organization sufficient to provide him with a general survey of existing know-

ledge on any subject within his sphere"; the second desideratum was to be satisfied by a "general research organization" coming under the Lord President of the Privy Council. This organization has gradually developed into five research councils but the responsibility for these was transferred from the Lord President first to the Minister for Science (1959) and later to the Minister for Education and Science (1965). One of the major advantages claimed by Haldane for his scheme was that "it places responsibility to parliament in the hands of a minister who is, in normal times, free from any serious pressure of administrative duties and is immune from any suspicion of being biased by administrative considerations against the application of the results of research". Although this independence of the "general research organization" from departmental influence and control disappeared in theory when the Lord President of the Council was replaced by the Minister of Education and Science, in practice the research councils decide their own programmes and it is this freedom to decide their programmes which will now be breached (for three of the present five research councils) if Rothschild's recommendations are accepted. Where Dainton, by various devices, hopes to make the research councils responsive to departmental needs "for scientific support" and thus to preserve their freedom, Rothschild says that "the executive departments should themselves ensure that they get what they want from the research councils and, if they do not, they can, and doubtless will, go elsewhere".

This is the crucial issue. Will the control of certain areas of government civil research be under a Board of Research Councils responsible to the Minister of Education and Science or will it be largely decentralized to the various departments as is already the case for industry and other areas? It is hard to escape from the conclusion that each department must carry the responsibility for its own research and development. How else can it be held accountable to parliament for failure to achieve any objective through scientific or technological incompetence? Can one imagine a minister explaining some such failure by saying that the Board of the Research Councils had not fully appreciated the importance of research on that topic and had considered the construction of another high energy accelerator more timely and promising? All attempts to establish non-political criteria by which to measure the relative priorities of scientific projects in different fields have failed.

There are, however, three objections to giving each department a free hand with its research and development. In

the first place this can lead to a great deal of wasteful duplication, especially as the research becomes more basic; second, the decisions on which problems should be tackled may be taken by people who have insufficient scientific background to make correct judgments on technical feasibility, and third, too little basic and strategic research may be done relative to the applied research and development. It is clear from the correspondence in the press that many scientists are especially concerned about the last two points if the Rothschild proposals are accepted. They prefer the Dainton report because they have great confidence in the research councils to prevent either or both of these mistakes. Actually the Rothschild proposals to transfer responsibility for part of the work of the ARC, MRC and NERC to the respective departments would be implemented gradually over a four year transition period so there would be no sudden diminution, if any, in the total budget of any of the research councils which are affected. It is the fact that a certain fraction of each of these three budgets is now to be earmarked for applied research which is causing some apprehension. No justification is given why that fraction should be 25 per cent (rising to 50 per cent) for the Medical Research Council. In the case of the Agricultural Research Council, a detailed justification of the budget split is given but even here one can foresee difficulties arising. Thus for Rothamsted and the John Innes Stations it is merely stated that a proportion of their work should continue to be regarded as basic and paid for by ARC. Is this fraction to be reviewed annually or quinquennially, and, if so, by whom?

Surely it would have been more sensible to transfer the whole of the ARC to the Ministry of Agriculture, Food and Fisheries, making the chief scientist of MAFF a full member of it. It was in fact such a proposal which stimulated the Council for Scientific Policy to produce the Dainton report. In short, Rothschild and Dainton each seem to me to propose awkward and probably unworkable compromises between the departments having full responsibility for all their required research and development and the research councils (with their associated new board) having responsibility for all government civil research, leaving only development and application to the individual departments.

A new framework is indeed needed for government research and development but it is an illusion to think the introduction of a customer-contractor principle for applied research and development can be a substitute for, or automatically lead to, a satisfactory new framework.

NEWS AND VIEWS

Binary Stars as Astronomical Accelerators

BINARY systems have always been of particular interest to astronomers. Both the mass and distance scales depend crucially on observations of binary stars, and because of the rapid evolution which can occur in such systems as a result of mass transfer between the components they also provide an insight into how stars age. Recently, information has begun to be gathered about binary systems at radio and X-ray frequencies, and there is a hint that gravitational radiation and the presence of black holes have roles to play in their evolution.

On page 270 of this issue of *Nature*, C. M. Wade and R. M. Hjellming report the detection of two "new" radio stars. This raises the total number of known radio stars with "normal" optical counterparts to four—the other two being Antares B and Cygnus X-1—and it is interesting that all of these are binary systems. But perhaps not too much should be made of this just yet, because the search carried out by Wade and Hjellming was inspired by the discovery of the nature of the radio emission from the Antares B system, so that they looked for similar emission from other binaries. The two systems newly observed at 2,695 MHz and 8,085 MHz are β Persei and β Lyrae, well known eclipsing binaries.

Cygnus X-1 can now be seen to provide a bridge between one class of X-ray variables and one class of radio variables. There is convincing evidence of the binary nature of the Cyg X-1 system (see page 271) and, although there is at present almost an embarrassing variety of X-ray variables, the variations of Cyg X-1 at X-ray frequencies are reminiscent of those of Sco X-1 at optical frequencies. Both show irregular variations, one feature of which is the presence of short wave trains with a more regular periodic variation, and in both relatively rapid flickering is superimposed on larger scale variations.

In the case of Cyg X-1, one explanation put forward to account for this behaviour involves the "ringing" of the star's magnetic field structure, oscillations being excited by flares occurring in regions of high magnetic field (G. R. Blumenthal and W. H. Tucker, *Nature*, **235**, 97; 1972). A similar idea has been proposed to account for some of the variations seen in the light from Sco X-1, but in this case the suggestion is that a flare excites acoustic vibrations in the atmosphere of the star.

Is there any evidence that Sco X-1 or any of the recently discovered X-ray variables is a binary? There is evidence that Cen X-3 has a periodical variation of ~ 2 days superimposed on its ~ 4.8 s X-ray period, and it is certainly desirable in general to invoke mass transfer from one member of a binary pair to the other as a convenient means of converting gravitational energy into X-rays. In the case of Sco X-1, the most intensively studied X-ray source, the evidence seems to favour a system more complicated than a simple binary, with perhaps a ring or disk of matter around the highly evolved star which is the X-ray source. Of course, this does not preclude the presence of one or more additional stars in the system as well. And Sco X-1 is a radio source, although the optical star associated with it could hardly be called normal.

One suggestion put forward last year is that Sco X-1 might be a similar system to the cataclysmic variable WZ Sagittae, a recurrent nova. This system consists of one star filling its Roche lobe, with mass being transferred from it first to a ring around the white dwarf companion and then on to the white dwarf itself. Such a system has obvious advantages if it is wished to invoke a mechanism which relates flaring and subsequent periodic structure in the variations of the flux from the source to the arrival of matter on the white dwarf component. But it is not just in the regime of X-ray astronomy that ultrashort period binaries of this kind (WZ Sge has an orbital period of 81.5 min) are of particular interest. It has been suspected for some time that gravitational radiation might be significant in the evolution of such systems, and it now seems, from a study carried out by J. Faulkner (*Astrophys. J. Lett.*, **170**, L99; 1971), that this is indeed the case.

Faulkner has developed a general model of such systems and applied it specifically to WZ Sge and Z Camelopardalis. He shows that losses by gravitational radiation can play a dominant part in the evolution of such a system, by removing both energy and angular momentum, and that a system which consists initially of two components each of mass $0.5 M_{\odot}$ will evolve by gravitational radiation on a timescale much more rapid than the timescale for nuclear burning of the main sequence component. The minimum binary period possible in such a system is about 30 min, consistent with observations, and it may be that these U Geminorum type systems evolve from W Ursa Majoris systems. The ejection of mass in successive nova outbursts may play a part in this, but it seems that energy and momentum losses by gravitational radiation alone can explain the evolution of a 10 hour binary into a 1 hour system. The obvious direct test of this theory is to measure the change in period caused by gravitational radiation. Unfortunately, however, this may not be practical in the case of WZ Sge because Faulkner predicts a change in phase of only 10 s in 30 years, and the two known outbursts of this object were in 1913 and 1946.

But the greatest interest in binary stars in recent months has perhaps been that evoked by the claim that there is now firm evidence that black holes are present in some such systems. Indeed, β Lyrae, one of the two new radio stars discovered by Wade and Hjellming, is one of the candidates for a binary with a black hole component (E. J. Devinney, *Nature*, **233**, 110; 1971; and R. E. Wilson, *Nature*, **234**, 406; 1971), and both β Lyrae and β Persei contain non-thermal radio sources. Another system which may contain a black hole is ϵ Aurigae.

According to R. E. Wilson (*Astrophys. J.*, **170**, 529; 1971), the earlier, dynamically implausible models for ϵ Aur, which involved rather peculiar disks of material orbiting the primary, can now be replaced by a more straightforward model. The peculiar features of ϵ Aur which any such model must explain are that the eclipse is flat bottomed, but that the primary remains spectroscopically visible throughout the eclipse. This implies that the bright component is not simply disappearing

behind a larger, dark companion. Eclipse by a disk of matter seems a promising way of explaining the observed phenomena, however, and Wilson's approach is the most fruitful yet.

According to this model, the disk of matter around ϵ Aur has a central opening rather like that of the rings of Saturn. Such a thin disk, orbiting in the plane of the binary system, could produce a flat bottomed eclipse because the progression of the eclipse may be compensated by the increase in the light seen through the central opening. Attractive as this idea is, it requires detailed calculations to confirm its plausibility. Wilson has constructed computer models of such a system in which the form of the 1929 eclipse of ϵ Aur can be exactly reproduced, even to the extent of small dips in the light curve of ~ 0.03 mag at the beginning and end of totality, if the central opening is semitransparent and passes 30 per cent of the incident radiation.

The 1956 eclipse of ϵ Aur was unusual in that it showed a central hump of 2.5 mag instead of a completely flat bottomed light curve. This, too, can be explained if the opening in the centre of the disk became more transparent between 1929 and 1956. Another peculiar feature of the 1956 eclipse was a drop of 0.2 mag, with the appearance of an eclipse, close to the time of conjunction. Wilson suggests that this event records the eclipse of the primary by the secondary, which should, on his model, have been plainly visible at the centre of ring opening at that time. The comparable 1929 event would have been masked because the opening was then less transparent.

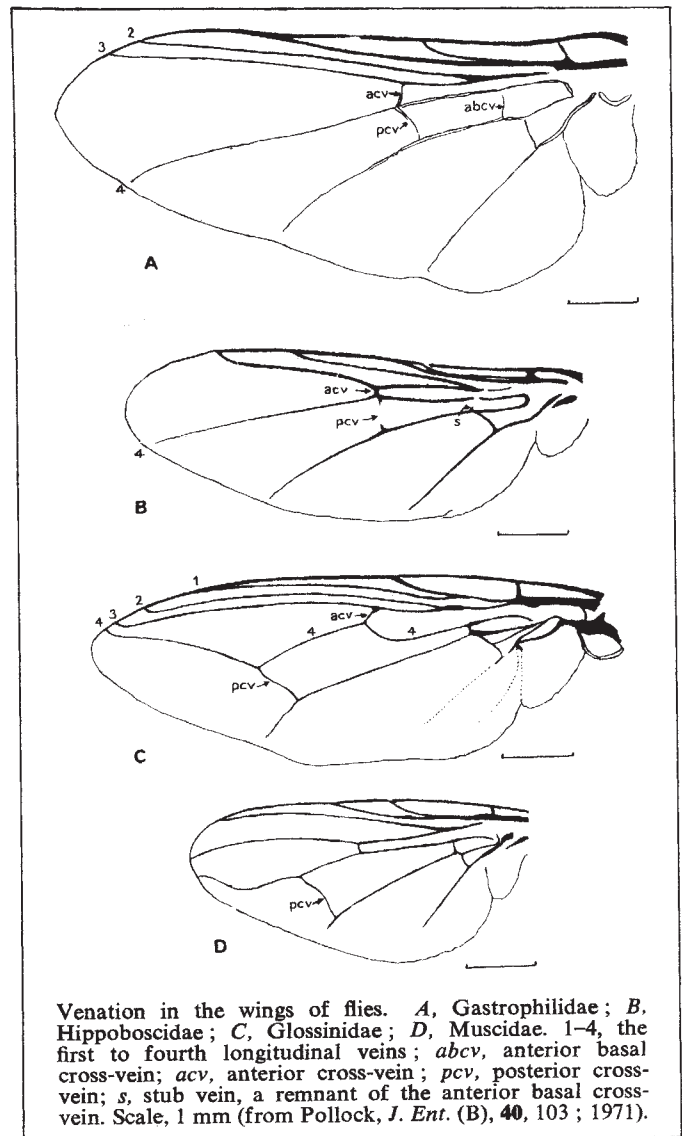
But what sort of secondary could produce such an eclipse? The duration of the event establishes the separation of the two components, and its magnitude gives some indication of their relative sizes. If the primary has the acceptable mass of $12 M_{\odot}$, it seems that the secondary, too, must be about $12 M_{\odot}$. An invisible companion of this mass could only be a collapsed object, presumably surrounded by a dust cloud some 0.3 a.u. in radius which produced the stellar eclipse. Such a cloud could be a transient feature, and its presence in 1956 might be directly related to the clearing of the opening in the ring system.

This work, together with evidence about binary systems now being gathered at all wavelengths of the electromagnetic spectrum (and possibly in the gravitational spectrum also), shows the wide diversity of ways in which natural phenomena are manifested. But in spite of these superficial differences, when it comes down to producing realistic models the physical situations seem remarkably similar. The real differences between the sources are differences of degree, not qualitative differences, and binary systems provide excellent opportunities to come to grips with the physics involved in high energy processes. So although binary stars have never been ignored by astronomers, it may be that their study is entering a new and profitable phase.

—From our Cosmology Correspondent.

Tsetse Fly Conservation?

THE tsetse flies—there are about twenty-two species of *Glossina*—have been called "Africa's bane". In this guise they have provided staple employment for a con-



siderable number of professional biologists during the past fifty years or more, and also a pretext for the deliberate destruction of Africa's endemic flora and fauna on a scale possibly surpassing the American defoliation campaigns in Vietnam. Few other insect genera can have occasioned the publication of so many large books, beginning with E. E. Austen's monograph of 1903, and ending with the recent work of J. Ford (*The Role of Trypanosomiasis in African Ecology*, Clarendon Press, 1971).

Within the past ten years or so, a belated awareness has dawned on some ecologists and conservationists that these same flies have been acting as the prime defence of Africa's endemic land vertebrates against invaders from other continents. The tsetse and the big game, it seems, may stand or fall together. The reasons for this situation are fairly easily understood. All the *Glossina* seem to serve as obligatory intermediate hosts for species of *Trypanosoma*, the alternate hosts of which are mammals, birds or reptiles. These trypanosomes as a rule have little effect on their usual African vertebrate hosts, no doubt as a result of long mutual adaptation, but are apt to be severely pathogenic to non-adapted non-African hosts. The flies themselves show little host specificity, and are as ready to suck blood from (and inoculate

trypanosomes into) recent immigrants as from their usual African hosts.

Quite apart from the value they may have in defending Africa, the tsetse flies have an evolutionary and systematic interest which few other insect genera could match. Systematically, the isolation of the genus was not at first realized, it having been originally regarded as near such familiar European types as the "biting housefly", *Stomoxys*, and in Ford's recent book it is still placed in Muscidae-Stomoxysinae. More recent students of Muscidae (for example, L. S. Zimin, in *Fauna of the USSR*, 18, part 4; 1951) have drawn attention to deep-rooted differences separating *Glossina* from *Stomoxys* or other Muscidae, and have proposed a separate family Glossinidae (another possibility is to include *Glossina* in a redefined Hippoboscidae), whose near relation to the ectoparasitic Hippoboscidae was indicated by Zimin and by J. C. Bequaert (*Entomologica Amer.* (n.s.), 34, 1; 35, 233; 36, 417; 1954-1957). This view is strongly upheld in an important recent article by J. N. Pollock (*J. Ent.* (B), 40, 101; 1971), who also makes the novel suggestion of a near relation of the *Glossina*-hippoboscid line to another isolated and enigmatic group of the higher Diptera, the Gastrophilidae.

Gastrophilid flies comprise two known genera, the adults of which are bee-like and hairy, very short lived, with vestigial mouth parts, and deposit eggs on the bodies of horses or rhinoceroses; the larvae live endoparasitically in the guts of these hosts. In *Glossina* and Hippoboscidae, on the other hand, feeding is purely an adult function; the larvae grow to full size within the body of the mother fly, and are deposited ready to pupate.

Pollock's suggestion of a relation of the *Glossina*-hippoboscid line to Gastrophilidae is supported by a new and controversial interpretation he offers of the wing venation of Hippoboscidae; this interpretation would make the *Glossina* wing derived from a primitive hippoboscid one rather than *vice versa*. Given that all the *Glossina* are fully winged and good fliers, with wing venation more like that of ordinary muscid flies, whereas hippoboscids are at best poor fliers, with a strong tendency to wing degeneration, it seems unlikely that the second group would preserve a more primitive wing venation. Most other features in which Pollock notes *Glossina* as resembling Gastrophilidae rather than Muscidae are likely to be primitive (plesiomorph) ones, and W. Hennig (*Phylogenetic Systematics*, University of Illinois Press, 1968) has warned of the danger of using symplesiomorphy as evidence of affinity. Gastrophilidae, it may be added, lack the principal diagnostic adult characters—a split second antennal segment, a "theca" sclerite in the proboscis and large squamae—of the housefly-blowfly group (Calypterae) of Diptera, all three of which are present in *Glossina*.

Even if Pollock is wrong in postulating a common origin for *Glossina* and Gastrophilidae, he produces cogent evidence for an early split between the glossinid and muscid lines. One striking piece of evidence for this is the occurrence in the Oligocene Florissant lake beds of Colorado of several species of fossils attributed to *Glossina* on account of their body form and of the unique type of wing venation. This not merely provides direct evidence of the antiquity of the type, but also suggests that it is likely to be even older. Existing *Glossina*, and the Florissant species if one is to judge from the asso-

ciated fossil fauna and flora, live in tropical or subtropical climates. It is probably necessary to go back to the Eocene period to find a New World-Old World connexion in a warm enough climate to be usable by *Glossina* (or monkeys!). A well authenticated fossil from the upper Oligocene of Rott, Germany (G. Statz, *Palaeontographica*, Abt. A, 91, 3; 1940), indicates the presence of specialized Hippoboscidae by that time, implying that Glossinidae, from which Hippoboscidae probably developed, must be considerably older than that. The oldest fossil attributed to Diptera-Calypterae is tachinid-like and is from the Upper Eocene of Wyoming; it thus seems that the glossinid-hippoboscid line was established very early in the development of Calypterae.

If Glossinidae lived in North America in the Lower Tertiary, they probably occurred then in Europe and Asia as well. The high degree of specificity in the *Trypanosoma*-*Glossina* relations today, and the fact that they involve trypanosomes of more than one subgenus, suggest that such relations are very old, and make it likely that American and other non-African Glossinidae of the past will have carried trypanosomes. It is thus quite possible that *Glossina*-carried trypanosomes may have formerly taken on other continents the kind of ecological role they now take in Africa. The presence or absence of *Glossina* in particular regions, or the spread of *Glossina* to new ones, may have had important effects on the development and spread of land vertebrates, including man's ancestors, during the Tertiary era.

Studies such as that of Pollock may eventually contribute significantly to understanding of the evolution of the higher Vertebrata as well as of the higher Diptera. Systematists and evolutionists would probably agree with those conservationists who consider the permanent survival of tsetse flies to be more desirable than their extermination.—R. A. C.

Immunoglobulin Structure

It is many years since it was first suggested that the recognition by an antibody of its antigen came about through a complementarity of their structures—a complementarity of topology, of electric charges and/or of polar and non-polar regions over a defined area of the molecular surfaces. The finding that an antibody raised against an intact protein molecule may not recognize the same protein molecule after it has been unfolded emphasizes that the spatial disposition of the groups forming the antibody-binding site is just as important as the nature of the groups. Thus, a complete understanding can only come about, as it has for enzyme specificity and catalysis, by a combination of the chemical experiments with a knowledge of the three-dimensional arrangement of the atoms of the binding sites of both the antibody and the antigen.

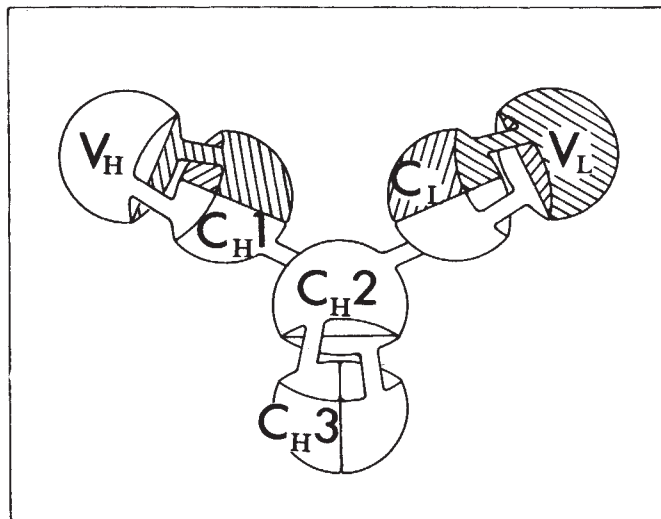
Quite apart from the fascinating problem of how the immune response is controlled, there is the puzzle of how the structurally similar members of the families of antibody molecules can have their recognition sites tailored to fit practically any antigen molecule that appears on the scene. The antibody molecules that have been studied most intensively are immunoglobulins of the IgG class. Such molecules, with a molecular weight of the order

of 15,000, have two identical halves. They may be thought of schematically as being in the form of a letter Y. The antigen-binding sites reside in the arms and the antibody is thus bivalent and can interact simultaneously with two antigen molecules. If the antigen molecules are themselves made of identical subunits, an infinite array or clump of them can be formed with the immunoglobulin molecules holding them together, so preventing the individual antigens from circulating freely in the system. A similar effect occurs even if the antigens themselves have no subunit structure because it is normal for a foreign molecule introduced into an organism to provoke the synthesis of more than one family of antibodies, complementary to different areas on its surface.

The consequence of the immunoglobulin molecule being Y-shaped and not a single compact globular unit is that it is flexible and this is probably a requirement if it is to function effectively. From the crystallographer's point of view, the flexibility is unhelpful; to build up a crystal it is necessary to have arrays of identical molecules and the flexible immunoglobulin molecules are apparently unwilling to form themselves into serried ranks. A second difficulty arises because in a normal organism even one class of immunoglobulin contains very many different molecules, alike in their general size, shape and physical properties but differing in the vital antigen binding sites; from such a population it is extremely difficult to separate out a homogeneous fraction containing molecules of one sort only which is of course required for crystallization to be possible. In patients suffering from multiple myeloma, however, the control systems of the affected cells go awry and in some cases the cells produce vast quantities of a single species of antibody. In such circumstances, it is much easier to separate and purify a homogeneous sample of molecules which there is no reason to doubt represent one of the antibodies that are normally present in controlled and modest amounts. (There is, of course, some difficulty in establishing the antigen to which such antibodies are complementary.)

Chemical studies of IgG molecules (see, for example, R. R. Porter in *Basic Problems in Neoplastic Diseases* (edited by A. Gellhorn and E. Hirschberg) 177, Columbia University Press, 1962; S. Cohen and C. Milstein, *Advances in Immunology*, **7**, 1; 1967) have shown that each half molecule consists of a light chain (of about 25,000 molecular weight) bonded by disulphide bridges to a heavy chain of about double the length. From the point of view of the amino-acid sequence, the light chain consists of two halves, V_L and C_L , between which there are many sequence homologies; the heavy chain seems likewise to consist of four quarters again (V_H1 , C_H1 , C_H2 , C_H3) with many homologies between themselves and also with the light chain segments. One of the two sections of the light chain, C_L , and three of the four sections of the heavy chain, C_H1 , C_H2 , C_H3 , seem to be constant in sequence in each of the broad classes of immunoglobulins whereas the remaining section of each chain is highly variable between immunoglobulins specific for different antigens. One light chain and the half of each heavy chain that contains the variable segment form each arm of the Y, the leg being composed of the remaining constant halves of the two heavy chains.

The difficulties of extracting a homogeneous species of molecules and then of persuading the essentially flexible molecules to conform to the regularity required to build



Poljak *et al.*'s schematic representation of the IgG molecule. Only one of the possible orientations of the Fc subunits relative to those of Fab is shown.

up a crystal have added considerably to the magnitude of undertaking an X-ray crystallographic study of molecular structure. In order to get round the problem of trying to grow crystals of intact molecules, many workers have used preparations in which the molecules have been broken down into component parts. Proteolytic enzymes may be used to cause specific cleavage of the polypeptide chain near the "joint" of the Y. This results in separating the arms (Fab fragments) from the leg (Fc fragment). The suffices indicate that the arms are found to retain the antigen binding capacity whereas the leg can be crystallized. Clearly the fraction that can most readily be crystallized is the least interesting to look at because it does not bind antigen and attempts to do so have perhaps consequently been rather half-hearted.

A second mode of cleavage in a different position gives rise to a slightly shorter leg and correspondingly longer arms known as Fab' fragments. It is of the latter fragments that Poljak, Amzel, Avey and Becka from the Johns Hopkins University and Nisonoff from the University of Illinois have now reported the results of a low resolution X-ray diffraction study (*Nature New Biology*, **235**, 137; 1972).

Other groups of crystallographers are also active in the field, as is only to be expected. Fortunately for all those involved in such long-term research, the first immunoglobulin molecule to be seen at atomic resolution will not yield by any means all that is to be known about the way in which the variations in amino-acid sequence between different immunoglobulins lead to such subtle differences in antigen specificity; such information will be forthcoming only when a number of different antibodies, together with their antigens, have been seen and compared.

Sarma, Silverton, Davies and Terry (*J. Biol. Chem.*, **246**, 3753; 1971) have been able to prepare crystals of a whole IgG molecule and have published a 6 Å resolution study which reveals two distinct types of ellipsoidal subunit which presumably represent respectively an arm (Fab fragment) and a leg (Fc fragment) of the molecule. As is common with low resolution maps, there are bands of density connecting these subunits together and it is not possible to tell which bands indicate polypeptide chains

crossing from one subunit to the next and which arise by chance proximity of chemically distinct groups when packed in the crystal. It is consequently impossible to deduce unequivocally which Fab fragments belong to the same molecule as which Fc fragment. The most likely combination, judged on the basis of the fewest chance connexions and the most dense bands representing real connexions, yields a molecule in which the subunits are joined in the expected Y shape. This work has revealed more precise overall dimensions for the fragments than had previously been available, but unfortunately Sarma *et al.* are not confident that the density variations within each subunit can be interpreted to give fine structural details. Sadly, the crystals of this molecule are rather disordered and the X-ray pattern becomes too weak for the analysis to be extended to higher resolution; indeed it is a tribute to the group's experimental skill that they were able to go as far as they did.

It is possible that the structure of the whole molecule will eventually be found by combining detailed studies of the individual fragments with lower resolution observations of the intact molecule. Certainly, the 6 Å maps of the Fab fragments obtained by Poljak and his colleagues go somewhat further. The resolution is too low for the polypeptide chains to be followed unequivocally or for the antibody binding site to be identified at all closely, but they are able to throw some light on one important question: is the similarity of amino-acid sequence between the two halves of the light chain and the four quarters of the heavy chain reflected in similar conformations for these regions of the molecule?

Poljak and his colleagues find that the density corresponding to each Fab' fragment is quite clearly delineated and that it is divided into two fairly distinct globules. The question now to be asked is whether one of these globules represents the light chain and one the arm half of the heavy chain, or whether both chains run through both globules. Several pieces of evidence, notably the fact that the variable regions of both the heavy and light chains are involved in the antigen-binding site and must therefore be close together in space, suggest that the first of these alternatives can be ruled out and this conclusion is supported by there being two distinct threads of electron-density that pass through both globules.

The situation seems to be therefore that the chemical similarities between the two halves of each chain are indeed reflected in conformational similarities, the two variable halves making up one globule and the constant halves the other. Poljak and his colleagues extra-

polate from this conclusion and suggest that the leg (Fc fragment) of the intact molecule also is composed of two globules, similar to each other and to the Fab globules, each built up from one of the two remaining constant regions of the two heavy chains (see figure).

As far as the Fab fractions are concerned, it is to be hoped that Poljak's group will be able to extend their analysis to higher resolution and, in particular, to see the antibody binding site in more detail. At present, the best chance of seeing the intact molecule appears to rest with Edmundson and his colleagues (A. B. Edmundson, *Cold Spring Harbor Symp. Quant. Biol.*, **36**; in the press) whose crystals give promise of permitting an eventual resolution of about 3.5 Å.—A. C. T. N.

MARS

Red Planet is not Dead

by our Cosmology Correspondent

PRELIMINARY results from the Mariner 9 science programme have now been published (*Science*, **175**, 293; 1972). The most important feature of these results and the television pictures sent back to Earth is that they show clearly that the red planet is far from being dead, and that weathering and volcanic activity are taking place on Mars to a significant degree. The most obvious feature of the planet's active state has, of course, been the large dust storm which

hampered observations just after Mariner 9 arrived in orbit around Mars. This has now cleared, and enabled good pictures showing many intriguing new features to be returned to Earth (see Figs. 1 and 2).

Ultraviolet and interferometric observations are consistent with an atmosphere composed chiefly of carbon dioxide, but both water vapour and the dissociation products H and O are also present. Comparison with the earlier Mariner observations, made in 1969, gives some idea of how rapidly Mars is changing. Mean tropospheric temperatures, for example, are now rather higher, perhaps because of the presence of dust in the atmosphere, and the south polar cap is much more regular than in 1969. Features have also emerged from the frost covering since Mariner 9 arrived, indicating that the cap is only a few centimetres thick.

Because of the dust storm and the residue of dust which it has left in the atmosphere, it is not yet possible to ascertain just how typical are the atmospheric observations made to date. In many ways, of course, this storm has been a blessing, because the resulting change in atmospheric properties over a period of months will provide a better insight into its composition and other features than repeated observations of undisturbed atmosphere always in the same dynamic state. But at present the most exciting information gleaned by Mariner 9 concerns the topography of the Martian surface.

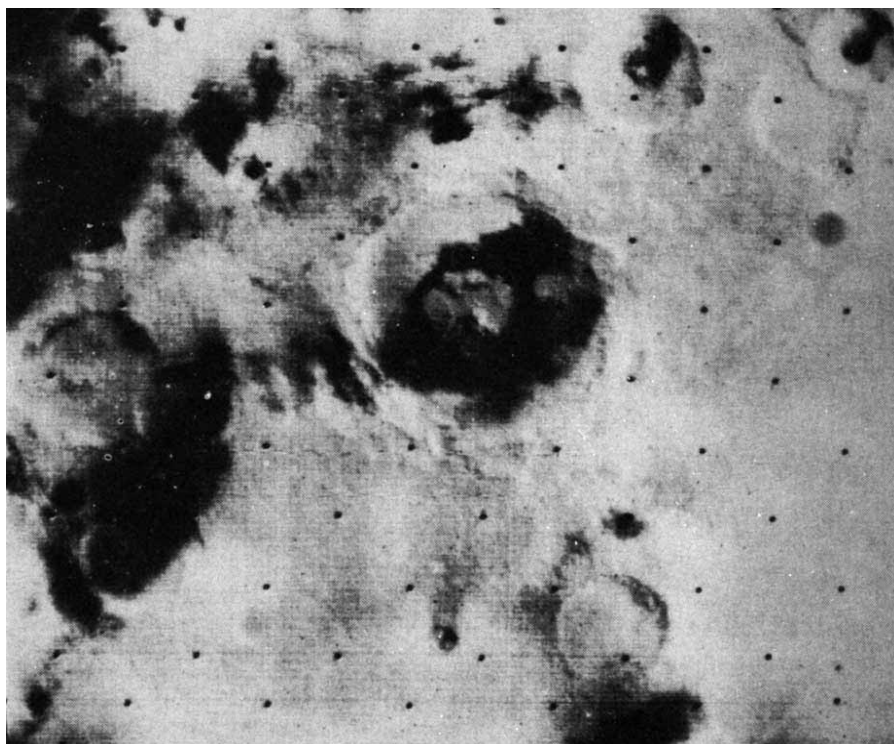


Fig 1. Irregular dark areas on the surface of Mars. These splotches have not been explained, but the largest are several hundred kilometres across, and may be related to "dark nuclei" reported by some observers on Earth when viewing conditions are ideal. The crater at the centre of the picture is roughly 125 km in diameter.

Although there is a superficial resemblance between the cratering of Mars and that of the Moon, in many cases the morphology of the Martian craters is quite different from that of lunar craters. The dark spots known as Nix Olympica, North Spot and South Spot are particularly interesting. Each spot contains a crater or crater complex, the Nix Olympica and North Spot four or five overlapping craters each. These seem to have formed successively, have very low rims, and do not have surrounding sharp ridges like those of most lunar craters. South Spot is a single structure roughly 100 km across, and unlike any known terrestrial or lunar crater. Several small rimless craters are seen on its rim, and some of these form slightly bent chains. Major valleys extend to the north-east and south-west of the South Spot, and it may not be a coincidence that this is also the direction of alignment of the North Spot, Middle Spot and South Spot.

In all of these spots there is morphological evidence of subsidence, and the broad features are reminiscent of volcanic calderas. It is difficult to explain their appearance as the result of meteoritic impacts. Also, the sharpness of the features and the smoothness of the surrounding terrain suggest that the spots are geologically young features, contrasting with the heavily cratered areas observed by earlier Mariner probes. The striking pictures of Phobos and Deimos also obtained by Mariner 9 provide a control by which the effects of weathering on Martian craters can be estimated, and these confirm that Mars is very much an active planet.



Fig. 2 Erosion pattern in the walls of a 50 mile wide down-faulted valley on Mars. Subsidence caused by volcanism and wind erosion might have caused the weathered appearance, which resembles scouring patterns produced by occasional heavy rain in arid regions of Earth. Arrows mark a crater chain like those found on the Moon. This may be the result of venting through a fracture in the crust.

VISION

Dark Happenings

from our Molecular Biology Correspondent

ASIDE from recent alarms and excursions, which are now known to have been fruitless, concerning a supposed covalent association of retinal with lipids, the train of chemical events in the bleaching of rhodopsin has stood solidly established for some time. The full horse-power of effort and conjecture has centred on the mechanism of excitation that follows this process. The question of regeneration of the rhodopsin in preparation for the arrival of the next quantum has by contrast received much less attention. Now, however, some attributes of this phase of the visual cycle have been uncovered.

In the first place, Shichi (*J. Biol. Chem.*, **246**, 6178; 1971) has confirmed that rhodopsin requires at least some of its bound lipid to function in this

respect. The protein resides, of course, in the membranes stacked in the rod outer segment, and as extracted in its functional state remains associated with a substantial amount of lipid. In intact rod outer segments, the ability of the pigment to regenerate is impaired by solvent extraction of lipids, and annihilated by treatment with phospholipase. If a solvent extract of lipid is restored to the depleted rod, the capacity to regenerate is regained. Moreover, single phospholipid components out of bottles serve just as well, but neutral lipids are ineffective. A simple interpretation of these effects is that the anionic phospholipids bind to the opsin, and exert a conformationally stabilizing effect (just as, for example, the native conformation of serum lipoproteins is disturbed by removal of lipid and restored when endogenous or extraneous lipids are added back).

Such an explanation is evidently on the right lines, for either phospholipase

Carbon Chemistry of the Lunar Surface

THE search for complex organic molecules in samples of the lunar surface brought back by the Apollo landings has given way to a study of the traces of light hydrocarbons and carbides which seem to be indigenous to this material. The low levels of total carbon (10–200 p.p.m.) and carbon compounds such as methane (0.1–5.0 p.p.m.) found in these samples require the development by organic geochemists of refined techniques for the handling and analysis of lunar material. Two of the university groups engaged in these lunar carbon studies are the Organic Geochemistry Unit at Bristol and the Space Sciences Laboratory at Berkeley, California, and in next Monday's *Nature Physical Science* (February 7) there are complementary contributions from these two laboratories.

Previous work by Eglinton *et al.* (*Nature*, **231**, 29; 1971) on Apollo 11 and 12 lunar fines suggested that traces of indigenous methane were probably derived chiefly from solar wind implantation, with minor contributions from lunar primordial sources. Lunar carbides or "carbide-like" material thus seemed to be of extra-lunar origin with contributions from both the solar wind and debris from meteoritic impact, the second probably being of lesser importance. The Bristol group now report the study of the simulation of solar wind bombardment of various lunar samples and metallic surfaces.

Targets of the various materials were irradiated in an isotope separator by ionized pieces of carbon ($^{13}\text{C}^+$) and deuterium (D_2^+) at energies similar to those estimated for the solar wind. The isotopically labelled species released by

acid treatment or pyrolysis of the implanted targets were then examined by mass spectrometry. Methane from the irradiated lunar fines was shown to be synthesized from the bombarding ions, and material reacting as carbide was synthesized in the metal targets. These simulation experiments also indicated that the meteoritic contribution to lunar "carbides" may be greater than previously suggested.

The studies of the Berkeley group are of particular interest because they include an examination of an Apollo 14 sample taken in the Special Environmental Sample container which was sealed on the Moon and not opened until inside the University of California's ultra clean handling facility. Gases released from this and other Apollo 14 samples by deuterium-labelled acid etching treatment showed that the amount of methane and neon and argon derived from the solar wind were related to grain surface area, confirming previous results for Apollo 11 and 12 fines. The pattern of evolved deuterium labelled hydrocarbons was similar to that produced from carbides, and grains of carbides have now been identified in lunar fines. This observation, taken in conjunction with calculations of the likely amount of carbon from solar wind bombardment, again suggests an important contribution from meteoritic material, supporting the suggestions of the Bristol group. The high degree of ^{13}C -isotopic enrichment observed in lunar fines might therefore result from fractionation during vaporization and deposition of meteoritic carbide, as well as from the "hydrogen stripping" process.

treatment of the micellar digitonin extract of rhodopsin, or prolonged standing of such preparations, which is accompanied by liberation of phospholipid, leads to a noticeable conformational change, as judged by circular dichroism in the peptide region of the ultraviolet, when bleaching occurs. In the absence of such treatment, Shichi finds (contrary to early reports) that the protein conformation is unchanged. A detrimental effect on both the regeneration capacity and the stability of the conformation is engendered by a nonionic detergent, now finding wide currency in rhodopsin work, with the sinister name of 'Emulphogene'. Shichi seems to envisage that the phospholipid is directly involved in stabilizing the stereochemically specific association of the protein with its prosthetic group, the 11-*cis* retinal. It is also, of course, to be expected that the apoprotein will be conformationally less stable than unbleached rhodopsin, and that the removal of further stabilizing ligands, that is to say the phospholipids, will permit more extensive unfolding, and render the molecule unable to return to the native state, so as to trap the 11-*cis* retinal for regeneration.

A very interesting observation, which, however, at present has no obvious place in the canon of retinal chemistry, is reported by Kühn and Dreyer (*FEBS Lett.*, **20**, 1; 1972). They have discovered a light-stimulated phosphorylation of rhodopsin in outer segments by ATP. This is not, it should be noted, a photochemical reaction, for it will work when ATP is added after a flash. Moreover, the extent of phosphorylation is proportional to the length of exposure to light, all of which goes to indicate that it is only the bleached opsin that is susceptible to attack. The transfer of the γ -phosphorus label to the protein shows that one is indeed dealing here with the introduction of a phosphoryl group: when the label is in the α -phosphorus, no radioactivity is incorporated, neither can the label in the protein be extracted or be chased by cold ATP. The maximum extent of phosphorylation is about one mole per mole of protein. Acid or enzymatic hydrolysis shows that the label is in the form of phosphoserine, and to a lesser extent phosphothreonine, which may be generated by transphosphorylation during the hydrolysis.

The phosphorylation reaction requires magnesium ions, and will not proceed after the membrane has been disrupted by 'Emulphogene' or a cationic detergent, even though these do not denature the rhodopsin. This evidence points to the participation of a membrane-bound kinase in the reaction. Kühn and Dreyer state that there is no detectable stimulation by cyclic AMP, so that any

scheme based only on a cyclic AMP-dependent kinase is ruled out (even though adenyl cyclase has been reported to be present in the rod outer segment, and is controlled—albeit inactivated—at some remove, by light). One can at this stage only speculate about the possible part of phosphorylation in the regeneration process, but the observations should stimulate some fast thinking.

Regeneration at a different level—the resynthesis of opsin—has been studied *in vitro* in retinas by O'Brien, Muellenberg and de Jong (*Biochemistry*, **11**, 64; 1972). This represents an advance on earlier work which was confined to living animals. On incubation with radioactive leucine, the label appears, after a lag phase, in the rhodopsin, as well as in another component that is chromatographically

identical with opsin. It is cautiously suggested that this might be a rhodopsin precursor, and indeed its concentration relative to labelled rhodopsin diminishes with time. Taken at face value, this would most simply be interpreted to signify that freshly synthesized opsin, synthesized in the base of the rod, migrates into the outer segment, there to meet its retinal and be converted into functional rhodopsin.

It may be noted that the wrangle concerning the molecular weight of rhodopsin still continues. On the basis of amino-acid analysis and detergent-gel electrophoresis, Robinson, Gordon-Walker and Bownds (*Nature New Biology*, **235**, 112; 1972) opt for the high value of 40,000. In spite of the hazards of applying detergent-gel calibrations to glycoproteins, this is the value on which most workers now

History of the Corsica-Sardinia Microplate

FROM the point of view of plate tectonics the Mediterranean is a complicated part of the world, being a region of numerous small interacting plates (microplates) rather than a part of one large one. But as Alvarez points out in next Monday's *Nature Physical Science*, complexity or not, the boundaries of the plates must be determined and their motions plotted. Accordingly, he has taken a step forward by trying to trace the history of the Corsica-Sardinia microplate and its relations with its near neighbours, France and Italy.

There now seems little doubt that in the course of time Corsica-Sardinia has rotated to its present position from a region adjacent to France. There is also another view which says that the deep sea (Ligurian Sea) between Provence and the present Corsica-Sardinia resulted from the subsidence of a former landmass—and there is even a little ambiguous evidence for it. But the evidence for the rotation hypothesis is stronger and unambiguous. Thus Corsica makes a good fit with France along the 1,000-metre isobath, which enables Permo-Carboniferous outcrops in Corsica to be matched with similar outcrops in Provence and leads to the alignment of the offshore circular magnetic anomalies thought to result from andesitic volcanoes. Moreover, the rotation is consistent with palaeomagnetic data based on Permo-Carboniferous rocks from Corsica, Permian rocks from Sardinia and Oligocene-lower Miocene rocks, also from Sardinia. In each case the palaeomagnetic directions differ from those of the mainland by the right magnitude.

The chief problem thus seems to be not whether rotation has occurred but when and for how long. Suggestions for the time of rotation have ranged

from Triassic to Miocene; but in Alvarez's view the later date is the correct one—and this he sets out to prove. In this he follows De Jong *et al.* (*Nature*, **224**, 67; 1969) whose palaeomagnetic data indicated rotation by the lower Miocene volcanics of Sardinia, thus implying that the rotation must have taken place since these rocks were emplaced.

Part of the new evidence adduced by Alvarez concerns the valleys near the coast of Provence which apparently drained northwards during the mid-Miocene. Because these valleys now contain crystalline pebbles untypical of the area, some workers found the need to invoke a crystalline massif off the coast of Provence which later subsided. But not only is there no seismic evidence for this, such a massif would have had to be extremely narrow (the coast of Provence is no more than 20 km from the abyssal plain), which is unlikely, or to have mysteriously sunk into the deep ocean, which is equally unlikely. It is more reasonable to suppose that the pebbles come from Corsica-Sardinia which, if the Miocene rotation hypothesis is valid, would have been in the right place at the right time. This being the case, evidence from dated fossils also found in the Provence valleys shows that Corsica-Sardinia could not have separated from France before 11 or 12 million years ago. The end of the rotation, on the other hand, is limited by the recently-discovered salt domes under the Ligurian Sea floor which seem to be derived from upper Miocene evaporites. Alvarez deduces from these features that rotation must have ended soon enough to give the evaporites time to be deposited during at least a part of the Messinian period (7–5 million years ago).

seem to be homing. Heitzmann (*ibid.*, 114) arrives at 35–40,000 on similar grounds. If this is correct, it follows that some 80 per cent of the total protein of the rod outer segment is rhodopsin.

NUCLEIC ACIDS

Modification Methylase

from our Cell Biology Correspondent

THE problem faced by organisms of distinguishing self from non-self has, of course, led to the emergence and diversification of the immune system; it has also resulted in the evolution in bacteria, and perhaps elsewhere, of host restriction and modification systems which prove to be sets of enzymes capable of marking and distinguishing indigenous DNA from invading DNA. Since their discovery the question of how bacterial modification and restriction enzymes work has attracted much attention and, thanks to the efforts of several research groups, there is now a clear overall picture of the process.

It seems that, in any particular strain of bacteria, DNA after replication is modified by the methylation of a quite small number of bases at specific sites in the molecule. A specific modification methylase catalyses the reaction and there is ample evidence which indicates that it uses S-adenosylmethionine as a source of methyl groups. As a result of its methylation the DNA is rendered resistant to the cell's restriction endonuclease, most probably because the methyl groups mask the sites in the DNA which the endonuclease specifically attacks. By contrast, invading DNA from another strain, either unmodified or modified to a different pattern, is unprotected and therefore is accessible to the restriction endonuclease which degrades it. Modification and restriction enzymes, in short, provide the bacterial cell with a defence against invading genomes.

Two reports in the *Journal of Molecular Biology* (63, 1, 9; 1972) by Smith, Arber and Kühnlein and Kühnlein and Arber leave little doubt as to the correctness of this overall picture and add some fine detail to it. Exploiting the fact that the single stranded DNA genome of coliphage fd contains very few methylated bases, Smith *et al.* have succeeded in measuring, by two methods, the number of N-6-methyladenine residues per genome in unmodified phage and modified phage grown on *Escherichia coli* B. They find, gratifyingly, twice as many methyladenine residues in the modified DNA as in the unmodified DNA; furthermore, when a mutant fd phage which is insensitive to B restriction is grown on *E. coli* B its DNA is not methylated at

the B modification site and so contains only half the number of methyladenine residues present in B modified wild type phage. The residual methyladenine residues in the mutant DNA presumably have no relation to the B modification restriction process. In short, B modification involves methylation of DNA and in the case of fd DNA complete B modification results in the methylation of one adenine residue at each of two B specific modification sites.

Paralleling these measurements Kühnlein and Arber have continued their investigations of partially purified *E. coli* B modification methylase, an enzyme which they have shown methylates *in vitro* double stranded replicative form fd DNA but not single stranded fd genomes. Methylation of replicative form DNA *in vitro* follows first order kinetics—all sites are equally efficient methyl group acceptors—and ceases when each replicative form molecule is saturated with four methyl groups. Moreover, the replicative form DNA of a mutant fd phage less sensitive *in vivo* to B restriction than wild type phage is saturated *in vitro* by two methyl groups and the replicative form of a double mutant, insensitive to B restriction, does not act as a substrate for the modification methylase. These findings, as well as supporting those of Smith *et al.*, indicate that the B specific modification sites on both the plus and minus strands

of fd DNA are methylated at equal rates and probably have symmetric base sequences, a situation for which the modification restriction system of *Haemophilus influenzae* provides a precedent.

That such a small change as the methylation of two adenine residues in an fd DNA chain of some 6,600 bases renders the DNA resistant to a restriction endonuclease indicates the high specificity of these nucleolytic enzymes, a specificity which will no doubt prove useful. Danna and Nathans (*Proc. US Nat. Acad. Sci.*, 68, 2913; 1971), for example, have used *H. influenzae* restriction enzyme to cleave form I DNA of simian virus 40, a closed, circular double stranded DNA, into eleven resolvable fragments eight of which are yielded in amounts equimolar to the original DNA.

As Danna and Nathans comment, with so many restriction enzymes available, and more no doubt awaiting discovery, it may well be possible by judicious selection of enzymes to cut the genome of small DNA viruses, such as SV40 or polyoma virus, into a set of overlapping fragments small enough for an attempt to be made to sequence them. In other words the analysis of the base sequence of a DNA tumour virus genome may be one of the bonuses of investigations of bacterial modification-restriction systems.

DNA Complementary to Globin mRNA

IN next Wednesday's *Nature New Biology* (February 9) there are reports by Baltimore's group and Spiegelman's group of the synthesis by reverse transcriptase isolated from avian myeloblastosis virus (the most convenient source of a reverse transcriptase) of a single stranded DNA molecule complementary to at least part of the messenger RNAs for rabbit and human globins. One of the first properties of reverse transcriptase to be discovered was its lack of rigorous template specificity; unlike the RNA replicases which replicate the genomes of the RNA bacteriophages and have extremely fastidious template requirements, reverse transcriptases are capable of using a variety of natural and synthetic RNAs as templates for DNA synthesis.

This finding immediately raised the possibility of using reverse transcriptase to make radioactively labelled DNAs complementary to specific messenger RNAs. Such DNA molecules, of high specific activity, might then be used as a probe for genes and gene products in nucleated cells. Globin messenger RNAs from various species, which have been partially purified, provide the obvious model system in which to test these ideas and several groups of re-

searchers began the obvious experiments.

As Verma, Temple, Fan and Baltimore and Kacian *et al.* are first to report, in the presence of short poly(dT) chains, which presumably act as primers, and in the presence of actinomycin D to restrict DNA synthesis to single strands, reverse transcriptase from avian myeloblastosis virus will indeed synthesize an 8S single stranded DNA when programmed with 10S globin messenger preparations from rabbit or human red cells. Furthermore, although, as Baltimore's group observe, it has yet to be rigorously proven that it is the active globin messenger molecules that are serving as templates for this DNA, a variety of lines of evidence indicate that this is most probably the case.

In short, even if reverse transcriptase proves to be of little significance to cancer researchers out to cure or prevent that disease, it seems destined to prove an extremely useful reagent for molecular biologists faced with the task of unravelling the genome and mechanisms which regulate its expression in eukaryotic cells. To be able to make very hot DNA complementary to specific messenger RNAs is something of a pipe dream come true.

FLUORESCENT PROBES

Time Resolved Redshift

from a Correspondent

STUDIES on the conformation and polarity of biological enzymes and membranes have made increasing use of fluorescent probes in recent years. Although a large number of articles have appeared in which probe interactions with biological substrates were examined, comparatively few investigations have been concerned with the behaviour of typical fluorescent molecules in well-defined environments. A welcome addition to this last category is an important contribution from Ware's group (S. K. Chakrabarti and W. R. Ware, *J. Chem. Phys.*, **55**, 5494; 1971), who report temperature and time-dependent fluorescence measurements on the well known fluorescence label, 1-anilino-8-naphthylene sulphonate (ANS).

Chakrabarti and Ware are primarily concerned with the redshift of the ANS fluorescence band maximum in polar solvents. For a fluorescent molecule which is free to diffuse in solution, these spectral shifts are thought to arise from solvent reorientation about the excited state that is required to accommodate the transient changes in electronic structure which occur upon light excitation. (The excited singlet state of ANS is more polar than the ground state as a result of the charge-transfer nature of the spectral transition.)

Ware has developed an instrumental technique which can be used to measure the transient changes with time over the complete fluorescence spectrum. This makes it possible to study kinetically the dynamic processes which determine the spectral change, namely the solvent environment and its relaxation subsequent to excitation of the absorbing molecule.

Chakrabarti and Ware find that time dependent spectral shifts for ANS are observed only in the temperature range from -70° to -170° C in *n*-propyl alcohol. At higher temperatures they see the fully relaxed time independent spectrum normally found at room temperature, with wavelength maximum at 480 nm.

In general, the time required to approach the relaxed spectrum gets longer as the temperature is decreased or the viscosity of the solvent is increased. At temperatures less than -170° C, no time dependent spectral shifts were observed, and the fluorescence intensity maximum is blue-shifted to 438 nm. This indicates that under these conditions the time for reorientation of solvent molecules is too slow to affect the emission properties of the fluorescent species. In essence, each emitting ANS molecule is in an

environment with nearly the Franck-Condon solvent configuration.

These results are quite similar to those reported earlier for the 4-aminophthalimide-propyl alcohol system (W. R. Ware *et al.*, *ibid.*, **54**, 4729; 1971), except for the spectral behaviour at very short times after excitation. The fluorescence spectra of the aminophthalimide showed evidence of a sub-nanosecond relaxation process which occurred even at low temperatures. The rapid effects were discussed in terms of interactions within an electronically excited solute-solvent complex.

These time dependent measurements emphasize the ambiguity which exists when spectral shifts of ANS or similar molecules are used as probes for the polarity of their surroundings. Only when "molecular motion akin to solvent reorientation" occurs will the probe function reliably as a polarity indicator. Specifically, interpretation of experimental results is expected to be more difficult when ANS is bound to a biological substrate. Some binding sites may exclude solvent molecules (see S.

Ainsworth and M. T. Flanagan, *Biochim. Biophys. Acta*, **194**, 213; 1969), and others may place geometrical constraints on the probe, thereby changing its emission properties. It will be interesting to see if time resolved emission spectroscopy can be used to simplify the interpretation of spectral shifts demonstrated by fluorescent probes.

RADIOLOGICAL PROTECTION

In the Event . . .

from a Correspondent

THE problems of radiation exposure associated with nuclear weapons were discussed at a meeting of the Society for Radiological Protection at Imperial College, London, on January 11. The characteristics and effects of nuclear weapons were described by Mr J. C. Cotterill (Home Office) who considered that the immediate damage by radiation exposure from medium and high explosive nuclear bombs would be within the range of destruction created by the blast and thermal effects. But radio-

B and T Cells Separated

VARIOUS means have been adopted to produce populations of cells in which the proportion of B and T cells has deliberately been adjusted. In earlier, cruder, experiments lymphoid cell populations from either neonatally thymectomized or adult thymectomized and irradiated mice have been taken to be selectively depleted of T cells and therefore rich in B cells. But it is now realized that there are many residual T cells in these animals and that their use is thereby restricted.

The discovery and description of athymic (Nu/Nu) mice provided a source of very nearly pure B cells, but this particular strain of animal is both difficult to rear and is at present not inbred. Miller and Sprent (*Cell. Immunol.*, in the press) have devised a more esoteric system whereby CBA thoracic duct lymphocytes are injected into heavily irradiated CBA $\times$ C57Bl mice. The cell population which, subsequently, can be recovered from the thoracic ducts of the recipients is a nearly pure T cell population but is also heavily committed to an anti-C57Bl response. Perhaps the best method so far has involved the use of anti- θ antisera which are capable of killing T cells in the presence of complement. The use of such selective sera has, however, been criticized on the grounds that they may sometimes contain anti-B cell antibodies, autoantibodies, and fail to eliminate transformed T cells. Basten *et al.*, in *Nature New Biology* next Wednesday (February 9), now provide an elegant method for the separation of B

and T cells which should be generally applicable to normal mice in any reasonably well-equipped laboratory.

It was first established that, when mouse thoracic duct lymphocytes were incubated sequentially with either mouse anti-fowl gamma globulin or mouse anti-human gamma globulin and then with either iodinated FyG or HGG, 15 to 20 per cent of the cells bound the radioactive material. An autoradiographic method was used to show this. As expected, cells incubated with anti-FyG only bound FyG and cells incubated with anti-HGG only bound HGG. Evidence is presented that the binding depends on the formation of antigen-antibody complexes on the surfaces of the binding cells. Basten *et al.* go on to show that, if they pass immunoglobulin coated cells through columns of beads themselves coated with the appropriate antigen, selective retention of those cells which then form surface antigen-antibody complexes can be achieved.

Basten *et al.* demonstrate clearly that they can remove B cells from a lymphoid cell population. They conclude that B cells have weak receptors for immunoglobulin molecules. Formation of an antigen-antibody complex can strengthen the tie between an attached immunoglobulin and its carrier B cell in such a way that the bead-antigen-antibody-cell complex is firm enough to withstand the forces of gentle elution. Subsequent discharge of B cells from the column was achieved by vigorous shaking with an appropriate medium.

active fallout would be produced by a groundburst, which drags up earth into the fireball to be irradiated in a molten state and, more important, forms a vehicle for the fission products. This fallout would cover a wide area and would constitute the longer term radiation hazard from a nuclear burst.

Dr R. H. Mole (MRC Radiobiology Unit, Harwell) discussed the radiobiological factors which enter into the assessment of an acceptable exposure under emergency conditions and pointed out the importance of accuracy in predicting the dose response. Considerations of tissue damage and patterns of recovery have suggested a simple formula for deducing lethal damage caused by a protracted exposure, and this may be used to control planned exposures of people who leave shelters temporarily for essential work or to decide when life outside may begin.

The British Government's plans for the organization of the administrative system for the survival and recovery of the country in time of a nuclear war were described by Mr V. G. Barry (Home Office), and his colleague, Mr G. A. Potter, outlined the British Warning and Monitoring Organization. The organization is responsible for originating warning to the public of the threat of air attack and the approach of fallout.

Dr H. D. Evans (Imperial College, London) described the task of the Regional Scientific Advisers and their associated scientific staff. The emphasis of their work is on health physics, but Dr Evans thought that their duties could well extend to advising on the use of resources following a nuclear attack.

Protection factors of buildings (which are ratios of dose in the open to that in the protected area of the building) were shown by Mr J. M. Reid (University of Glasgow) to extend up to 1,000. Obviously some of the protection is gained by the geometrical effect of spacing away from the external contamination, but the remainder is attributable to the absorption of radiation in the building materials of the structure.

The procedures used to evaluate exposure regimes were illustrated by Mr J. Sharpe (EMI Electronics, Hayes). He pointed out that the concept of recovery from an initial radiation dose and from lower daily doses allowed brief exposure of personnel to high dose rates for essential work. The same principle allowed determination of the times at which the general public could emerge from refuge for increasing daily periods.

The relatively neglected aspect of the hazard to farm animals was discussed by Mr A. A. Wilson (University of Cambridge). He pointed out that the bulk of the national herd would be in the open or in buildings offering relatively little protection.

Detection of Primate Cancer Viruses ?

LAST month, Todaro and his colleagues reported in *Nature New Biology* (235, 35; 1972) their investigations of the reverse transcriptases and other components of four C-type RNA viruses which are produced by cultures of primate cells. One of these four viruses was isolated from the cells of a gibbon ape (*Hylobates lar*) bearing a disseminated lymphosarcoma by Kawakami and four associates who, in next Wednesday's *Nature New Biology* (February 9), describe the detection of the virus.

Having observed under the electron microscope typical C-type particles in sections of lymph nodes, liver, spleen and bone marrow tissue from this ape, Kawakami and his colleagues established a relatively homogeneous culture of its lymphoid tumour cells and found that a C-type virus accumulated in the cell's culture medium. Bovine, simian and human cells in culture are susceptible to infection by this virus and support its replication but show neither morphological changes nor cytopathological effect.

After further purification, the virus was found to possess many of the diagnostic biochemical and biophysical characteristics of the vertebrate sarcoma and leukaemia viruses but it is anti-

genically distinct from known RNA tumour viruses. As Kawakami *et al.* say, although it has yet to be shown that this new virus has an oncogenic potential, both the circumstances of its discovery and its similarity to undisputed RNA tumour viruses make it a potential candidate primate RNA cancer virus, a conclusion Todaro and his colleagues no doubt fully endorse.

Another but quite unrelated primate virus which may have oncogenic potential is also reported in next week's *Nature New Biology* by Melendez and six associates. They have detected in primary cultures of kidney cells of an apparently healthy spider monkey (*Ateles geoffroyi*) a new herpesvirus which they call *Herpesvirus ateles*. This virus, which causes cytopathic changes typical of herpesvirus infections in cultures of several simian and human cell lines, is antigenically distinct from other known herpesviruses.

The possibility that *Herpesvirus ateles* might be oncogenic has to be seriously considered because of three marmosets inoculated with the virus two died after 28 days and were found to carry malignant lymphosarcomas and the third marmoset killed after 40 days also had a lymphosarcoma.

Target Cell Death in the Absence of Complement

THE concept that an appropriate antibody in the presence of complement could lyse a target cell, nucleated or otherwise, has been established for some years. Latterly it has become apparent that certain kinds of cells (probably T cells), if left long enough in contact *in vitro* with target cell monolayers, can acquire the capacity to kill. Excited macrophages have been shown to liberate materials which promote killing by otherwise innocuous lymphocytes of target cells that they have not previously encountered. These findings and others, in their variety, reflect the pressure to understand the precise mechanism of homograft rejection *in vivo*. Fakhri and Hobbs, in next Wednesday's *Nature New Biology* present findings which illustrate the complexities of cytotoxicity.

Fakhri and Hobbs injected a mouse plasmacytoma tumour into rats and subsequently cannulated the thoracic duct lymph. The lymph itself was capable of destroying the tumour cells both *in vitro* and *in vivo* in the peritoneal cavity of recipient mice. Separated 7S globulins from the lymph had no such cytotoxic capacity, neither did separated cells. The separated 19S antibody was effectively lethal but required complement to be so.

Fakhri and Hobbs go on to investigate the killing potential of 7S antibody and

cells in combination but in the absence of complement. They found that when target tumour cells were incubated sequentially with 7S antibody and non-immune lymphocytes rosettes formed consisting of tumour cells surrounded by lymphocytes. Rosette formation could be inhibited by addition of anti-human Fc- γ between coating the target cells with immunoglobulin and addition of the lymphocytes. Without added 7S antibody immune lymphocytes formed rosettes with about 36 per cent of tumour cells which the authors presume is caused by direct contact between B lymphocyte receptors and surface antigen on the tumour cell. Rosette formation under all circumstances was inimical to the survival of tumour cells.

Fakhri and Hobbs contend that their results support the hypothesis of Möller (*Transplant. Proc.*, 3, 15; 1971) that T cells carry a receptor for the Fc of certain immunoglobulins. They amplify this notion and suggest that the receptor is only for Fc that has become activated by attachment of the Fab of the same antibody to its target. In their current experiments Fakhri and Hobbs presumably suppose that the receptor-Fc complex by which the cells are bound in the "non-immune" rosettes is the activating stimulus for the T(?) cells.

CS and its Chemical Relatives

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This article surveys the principal aspects of the chemical properties of benzylidene malononitriles (BMNs) and their effects on and interactions with living organisms.

BMNs are the chief products of the condensation of benzaldehyde and its derivatives with malononitrile. BMN itself was prepared by Heuck¹ in 1895. The interest which has been shown recently in this group of compounds is largely due to the use of 2-chloroBMN (CS) as the active principle in numerous anti-personnel and riot control devices²⁻¹³.

Malononitrile is usually the common product of mild hydrolysis of BMNs¹⁴⁻¹⁶, and a brief consideration of the effects of the dinitrile on biological systems is therefore relevant. The chemistry of malononitrile has been reviewed by Freeman¹⁷, who listed its chief physiological actions and biomedical applications. The toxicity of the compound has been studied in varying detail¹⁸⁻²³; values for the LD₅₀ have been reported²¹⁻²³. Thiosulphate raises the toxic level several-fold^{21,23}. The breakdown of malononitrile has been investigated in animal tissues; cyanide and thiocyanate are formed²⁴.

The effects on nervous tissue of administering the dinitrile have also received attention, but the results are not always in agreement. Degenerative effects have been described in nerve cells²⁵⁻²⁷; adrenaline-blocking has been reported in the cat²⁸, while mitochondrial enlargement and fragmentation of Nissl bodies occur in spinal ganglion cells of the frog²⁹. On the other hand, increased levels of protein and nucleic acid have been detected in nerve cells of rats and rabbits in response to malononitrile^{30,31}. The compound enhances oxygen consumption and tends to restore nucleoprotein levels in the central nervous system³². It has also been claimed that the dinitrile stimulates the regeneration of nervous tissue^{33,34} but other workers have found no alteration in the nucleic acid levels of rabbit and guinea-pig brains after administration³⁵. The discrepancies between some of these findings may be accounted for in terms of different dosages. In this context, the effect of sustained sublethal concentrations of cyanide gradually building up in the body^{23,24} might affect the brain adversely (for example, see refs. 36, 37).

Syntheses and Structure of BMNs

BMNs are solid crystalline materials, high yields of which are readily obtained when concentrated solutions of malononitrile and the appropriate aldehyde are mixed. The reaction is usually slightly exothermic and crystals separate within a short time. The condensation is greatly facilitated by the presence of an organic base such as piperidine; most workers have made use of this mechanism (for example, refs. 14, 38-47), which has been studied in detail^{48,49} and reviewed¹⁷. Early workers used sodium ethoxide¹ or hydroxide⁵⁰ to provide basic conditions; various other compounds can also be substituted¹⁴. The reaction will proceed without catalysis⁵¹, but much more slowly¹⁴. CS itself was prepared by Corson and Stoughton¹⁴, from whose names the trivial appellation is derived; a modification of the synthesis has also appeared⁴⁵. The crystals have

a melting point of 95°-96° (ref. 14) and possess a very faint brownish-yellow tinge. The vapour pressure of CS is negligible at ordinary temperatures, which means that the airborne compound is almost entirely in aerosol form. The terminology "CS gas" is therefore erroneous and misleading. Treatment of α -cyanocinnamamides with phosphorus pentachloride and dry sulphur dioxide yields BMNs and compounds of the type $\text{ArCH}:\text{C}(\text{CN})\text{CCl}_2\text{N}:\text{PCl}_3$; decomposition by water produces BMNs⁵². Phosphorus pentachloride alone gives BMN from either α -cyanocinnamamide or α -amidocinnamamide¹.

Following the application of certain BMNs as cytotoxic agents against tumours^{53,54}, various other derivatives, which incorporate *bis*-(2-chloroethyl)amino groups⁵⁵⁻⁵⁸, a *bis*-(chloromethyl)pyrrolidine substituent⁵⁹ or a phenylazopyrimidine residue⁶⁰, have been prepared for the chemotherapeutic treatment of cancer. Other compounds have been synthesized for use as pesticides⁶¹, fungicides⁶² and insecticides⁶³.

The dipole moments of BMN itself⁴² and of certain derivatives⁶⁴ have been measured and evidence has been cited for polarity in the side chain⁴⁴. Patai and Rappoport¹⁵ have suggested that resonance with a cyanide group can give rise to the conjugated structure (2).



Formula (2) is further substantiated by the inability of BMN to react with ozone⁶⁵, by the fact that bromine tends to substitute in the aromatic nucleus instead of reacting with the $\alpha\beta$ double bond⁶⁶ and by the high molar extinction coefficients which many BMNs possess in the ultraviolet^{15,42,45,49,67,68}. This conjugated structure largely explains the high reactivity of BMNs, and also furnishes the key to many of the mechanisms of reaction in which these compounds participate. Most of the reactions described here no doubt involve the initial formation of an adduct which, depending on its stability, may or may not undergo subsequent decomposition or transformation.

Chemical Reactions

BMNs readily form adducts of diverse stability with many substances, and various reaction mechanisms have been proposed⁶⁹. Potassium^{14,70} and hydrogen⁷¹ cyanide react to give weakly acidic trinitriles. The rates of reaction of various BMNs, including CS, with cyanide have been measured and rate constants derived⁷². Addition products of BMNs with trialkylphosphines, including that formed by CS with triethylphosphine⁴⁴, have been isolated⁷³⁻⁷⁵. The influence of substituents in the aryl ring on the mechanism of the addition has been examined⁴⁴. In certain cases equilibrium constants and ΔH values have been derived; the authors concluded that these adducts possessed a zwitterion structure⁷⁴. Treatment with methyl iodide regenerates the original BMNs, and quaternary phosphonium iodides⁷⁶ are also produced.

The reaction of BMNs with Grignard reagents has been used to prepare intermediates for syntheses^{77,78}. Adducts with compounds of transition elements are also known. These include stable addition compounds of BMN with cuprous

halides⁷⁹. Copper-containing complexes have also been prepared from the condensation product of terephthaldehyde with malononitrile⁸⁰. Less stable adducts of BMNs with nickel have been described⁸¹.

The reaction of 4-nitroBMN with hydrazoic acid has been studied⁷¹. Addition compounds are also formed with isocyanates and with isothiocyanates, the reaction being base-catalysed⁸². The kinetics and mechanism of reaction of certain BMNs, including CS, with cyclopentadienylidenetriarylphosphoranes have been described, and third-order rate constants have been determined⁸³.

To the biologist, the most interesting groups of adducts are those formed with amines and thiols; substances in these two categories are among the least stable of the addition compounds. The participation of triethylamine in the hydrolysis of BMNs has been observed¹⁵. Factors which govern rates of formation of adducts from BMNs and amines have been discussed in detail; rate constants for the addition of *n*-butylamine to CS and to other BMNs have been derived⁸⁴. Constants have also been determined for the addition of other amines, as well as *n*-butylamine, to more than twenty BMNs⁸⁵. The influence of the solvent on the rate of reaction has been investigated; anhydrous dioxan was used in most systems, because the reaction in ethanol or water proceeded too quickly for measurements to be made. Activation energies were determined in some cases; the value is 5.4 kcalorie mole⁻¹ for CS and *n*-butylamine⁸⁵. Adducts from aniline and two toluidines with some seven BMNs, including CS, are too unstable to persist at room temperature⁴⁷. Complex condensation products have been prepared by refluxing BMN with *n*-butylamine and malononitrile in ethanol⁸⁶.

Similarly, equilibrium is rapidly attained in the addition reaction between *n*-butanethiol and BMN in chloroform, but the reverse reaction proceeds too quickly to permit isolation of a product (G. Feniak, H. L. Holmes and J. B. Reesor, unpublished observations mentioned in ref. 87). Equilibrium constants have been derived for the reactions between *n*-butanethiol and nearly thirty BMNs, including CS and other 2-substituted derivatives, in aqueous ethanol buffered by phosphate; reaction rates were too rapid to measure by standard methods⁸⁷. The formation of adducts *in vitro* between CS and various -SH compounds, mostly of biological origin, has been reported⁸⁸; the fastest rates observed were those with the disulphydryl form of lipoic acid, and with dithiothreitol. These addition compounds are probably unstable.

In the absence of detailed knowledge of the decomposition of BMNs in the body, the phenomenon of hydrolysis is necessarily important to the biologist. Just as the formation of BMNs is catalysed by bases, so their hydrolysis to malononitrile and the original aldehyde is accelerated by mildly alkaline conditions^{14-16,70}. Although the initial mechanisms are different, both the base-catalysed^{15,16} and the non-catalysed¹⁵ reactions are believed to proceed through an intermediate of the type ArCH(OH).CH(CN)₂. This intermediate, which has not been isolated, decomposes in stages which involve the initial loss of a proton, followed by cleavage to give the parent aldehyde and a highly reactive carbanion, ⁻CH(CN)₂; this is then rapidly protonated to give malononitrile⁸⁹. The formation of BMNs is thought to proceed through the same mechanisms acting in reverse^{48,49}. Base-catalysed hydrolysis has also been studied by other workers⁷⁰. The activation energies of certain hydrolytic systems have been measured¹⁵. Rate constants for the hydrolysis of more than twenty BMNs in aqueous ethanol buffered with phosphate have been derived⁷²; work with CS has also been carried out^{14,15,72}. BMN has been described as a very weak Lewis acid (see for example, ref. 90). Treatment of BMN with ethanolic potassium hydroxide in excess⁹¹ produces 2-amino-3,5-dicyano-4-phenyl-6-ethoxypyridine.

BMNs bearing a halogen substituent on the ethylene nucleus react with primary aromatic amines to give products⁹² of the type Ar(ArNH)C:C(CN)₂.

BMNs are stable in dilute acid¹⁵; vigorous treatment by strong acids followed by water tends to hydrolyse one or both nitrile groups to amides. Concentrated hydrochloric acid converts 3,4,5-trihydroxyBMN to the corresponding α -cyanocinnamamide⁴⁰. When a suspension of CS in glacial acetic acid is successively treated with hydrogen chloride and water²³, the product is α -cyano-2-chlorocinnamamide (G. R. N. J., C. Zauli and P. S. Diamond, to be published). The material obtained by similar means from BMN was at one time thought⁴⁴ to be ArCH(OH).CH(CN)₂, but the product seems to be an α -cyanocinnamamide (G. R. N. J., C. Z., and P. S. D.); these can also be produced from BMNs by treatment with warm concentrated sulphuric acid and water^{51,93}. Under these latter conditions CS yields α -amido-2-chlorocinnamamide (G. R. N. J., C. Z., and P. S. D.). Treatment of 2-hydroxy-BMN, which is in fact 3-cyanocoumarinimide, with dry hydrogen chloride forms 3-cyanocoumarin⁹⁴. BMNs are partly hydrolysed by concentrated hydrochloric acid in the presence of triphenylphosphine to give (1-aryl-2-carbamoyl-2-cyanoethyl) triphenylphosphonium chlorides; when these salts are refluxed in ethanol, α -cyanocinnamamides are obtained, and triphenylphosphine is regenerated⁹⁵.

Oxidation of BMNs by permanganate produces substituted benzoic acids¹⁴. The reduction of BMNs has been studied polarographically^{96,97} and is held to be a single wave, one-electron process⁹⁷. The half-wave potentials of nearly twenty BMNs, including CS and several other compounds substituted in the 2-position, have been determined⁹⁸. An excess of sodium borohydride, followed by successive treatment with acid and alkali, fully reduces one nitrile group in each molecule of 3,4-dimethoxyBMN; water adds across the $\alpha\beta$ double bond, producing α -aminomethyl-3,4-dimethoxyhydroxyhydrocinnammonitrile⁹⁹. BMNs are also active poisons of Raney nickel in the catalytic reduction of cyclohexene¹⁰⁰. The discovery that hypochlorite-containing bleaches augmented the irritant effect of CS¹⁰¹ led to the isolation of an epoxide of the formula (2)-Cl-C₆H₄-CH-CH(CN)₂ from the stoichiometric reaction between



the agent and sodium hypochlorite¹⁰². Bromine and nitrous acid generally substitute in the aryl nucleus, while leaving the $\alpha\beta$ double bond in the side chain untouched⁶⁶. The dimerization of BMNs has been observed¹⁴ and studied⁶⁹. Copolymers with styrene have been prepared¹⁰³⁻¹⁰⁶.

Human Exposure

With Ehrlich's theory in mind that coloured compounds which bear toxic chemical groupings possess therapeutic potential, Walter⁵⁰ prepared 4-dimethylaminoBMN and 2,4-dihydroxyBMN, but no description of trials with these compounds in human or animal subjects has appeared. As a result of successes in treating animal tumours with certain BMNs^{53,54,107}, 4-nitroBMN was given to human patients with advanced sarcoma or carcinoma, but without effect¹⁰⁸.

Early workers experienced a stinging action on the mucous membranes caused by contact with certain substituted BMNs^{14,51}. A detailed account of the immediate physiological effects of CS aerosols is set out in the first patent to cover the application as riot-control agents of derivatives which bore substituents in the 2-position⁶. Evaluations of the usefulness of CS in this context were carried out at defence establishments³⁻⁵. An interim report described the effects on workers in chemical plants of exposures to CS¹⁰⁹. Reference to unpublished data describing allergic contact dermatitis in such subjects has been made¹¹⁰. Sensitization has been ascribed to alterations in the antigenic profile of serum proteins, due to the formation of -SH adducts with CS⁸⁸. Large particles of airborne CS, of mass median diameter 60 μ m, are tolerated better than smaller particles (mass median diameter 0.9 μ m). The effect of the larger particles is predominantly on the eyes, whereas the smaller ones affect principally the respiratory tract¹¹¹.

CS produces erythema of the eye and severe conjunctivitis as well as intense burning of the skin¹⁰¹. The most incapacitating effects are felt in the respiratory tract and include coughing, burning of the throat and feelings of chest constriction^{6,101}. Heavy cutaneous exposure may result in erythema and vesiculation which resembles second-degree burns¹⁰¹. A more detailed study¹¹² reported a differential racial response resulting from exposure to thermally-generated aerosols of CS at high humidity and 37°. In Negroes, erythema, which set in after a few hours, was the most serious symptom. In Caucasoids, widespread blistering also occurred some days later. Scarring was apparent six weeks after exposure¹¹². Visual acuity is not appreciably impaired by CS, but subjects had difficulty in keeping their eyes open after the initial exposure¹¹³. Cutaneous reaction to CS has been discussed¹¹⁰.

Animal Exposure

The effects of BMNs on animals are comparatively well documented but, as in the case of human exposure, access to much of the information is difficult and some remains classified. In such a situation the literature cannot be covered in its entirety by an independent worker.

The earliest animal exposures involved cancer chemotherapy. A growth-retarding effect on transplanted tumours in mice by various BMNs has been reported^{53,54}. All the derivatives tested which bore substituents in the 2-position of the aryl ring, including CS, were observed to cause irritation; moreover the toxicities of the compounds were similar to that of malononitrile itself⁵⁴. The original intention had been that the administration of BMNs might permit the local accumulation of cytotoxic levels of cyanide in certain tumours where rhodanese activity was low¹¹³. The pattern of metabolism in normal and malignant tissue, however, bears no relation to the distribution of the enzyme^{115,116}, which in any case is unlikely to mediate conversion of cyanide to thiocyanate in the absence of thiosulphate. Other BMNs are inactive⁴⁶; those bearing *bis*-(chloroalkyl)amino substituents have been tested against tumours^{55,57}, but predictions about how such compounds or azopyrimidine-substituted BMNs⁶⁰ might exert cytotoxicity are speculative.

Almost all the remaining work on the toxicity of BMNs has been carried out with CS, although certain other 2-substituted BMNs, especially the 2-nitro derivative, are comparably effective as irritants⁶. The initial studies²⁻⁴ on which the selection of the 2-chloro derivative as an agent for riot control was based were not published at the time, but the toxicity of the compound was considered⁴. A comparative study of the toxicities of CS, *o*-chloroacetophenone (tear gas) and 10-chloro-5,10-dihydrophenarsazine ('Adamsite', DM) was carried out later¹¹⁷. LD₅₀ values in various species have been reported as follows: in rabbits, by intravenous injection, about 8 mg kg⁻¹ body weight¹¹⁸; in mice, by intraperitoneal injection, 35 mg kg⁻¹ (ref. 23); in rats, by the same route, 36 mg kg⁻¹ (ref. 88). Mice can tolerate up to 800 mg kg⁻¹ injected subcutaneously¹¹⁸, and dogs can tolerate 8 mg kg⁻¹ administered intravenously⁸⁸. In mice which had received thiosulphate after lethal intraperitoneal injections of CS, no histological changes were reported other than venous congestion²³. LC₅₀ values (the product of the exposure time in minutes and the concentration of the agent in mg l.⁻¹ required to cause fatalities among half a group of animals) are: rats, 32.5; mice, 43.5; guinea-pigs, 8.3; rabbits, 17.3; pigeons, 36 (ref. 118). The average particle size was 1.5 µm in these experiments. Moribund rats generally died one day after exposure, whereas other animals were still dying up to ten days later. Severe erythema of the eyes was observed, in addition to conjunctivitis; although all exposed animals were killed within five weeks, the authors stated that no permanent eye damage had ensued. Increases in the populations of goblet cells in both the conjunctiva and respiratory tract were found. Particles of CS caused necrosis at sites of impact in the gastro-intestinal and respiratory tracts. Occasionally, pulmonary oedema and haemorrhage of the

adrenal glands were noted, more particularly at high inhalation doses¹¹⁸. An LC₅₀ of approximately 57 mg min l.⁻¹ has been reported for the anaesthetized dog⁸⁸. Initially, increases in the blood pressure and respiration rate, mediated by the thalamus, were observed as a response to CS aerosols; the oxygen tension and body temperature sometimes dropped, and there was a marked fall in the pH of the blood. After making partial recoveries, the dogs died in respiratory distress two or three days later. The lungs of exposed animals showed pulmonary oedema, haemorrhage and atelectasis. Rats were exposed to CS aerosols at dosages of 40 to 80 mg min l.⁻¹. The surfactant activity of rinsings from lungs of animals which were later killed was depressed; the same effect was more marked in animals which died. There was evidence of lysosomal damage; levels of β-glucuronidase activity in the same rinsings were nearly twice as high in the killed animals as in the controls, and were five times higher in rinsings from rats which died⁸⁸.

The effects of CS aerosols at four dosages (2.7, 8.5, 28.5 and 80 mg min l.⁻¹) have been described in monkeys¹¹⁹. The most prominent lesions at the two lower doses were mild pulmonary congestion, bronchorrhoea, emphysema and atelectasis; these cleared after three days, but recurred one and four weeks after exposure. Bronchopneumonia was present one week after dosage at the third level. Lesions which resulted from exposure to the largest dose were sufficiently severe to be revealed by X-rays. Most survivors killed up to 30 days after exposure had emphysema, atelectasis and bronchiolitis.

When injected into the blood system of cats and dogs in very small doses (50 to 200 µg kg⁻¹), CS acts as a non-specific irritant and affects the blood pressure¹²⁰. Some evidence suggests that CS can trigger the appearance of bradykinin-like activity *in vivo* and *in vitro*⁸⁸. Sensitization to CS has been reported in guinea-pigs¹²¹ and an explanation has been suggested⁸⁸.

Metabolism and Pharmacology

Evidence from various sources suggests that a strong link exists between the metabolism of malononitrile and that of certain of its benzylidene derivatives^{18,21,23,24,54,88,108}. On the other hand, conclusive data that malononitrile is formed *in vivo* from BMNs have not been presented. The nature of the effects of the compounds is considered to depend critically on the chemical identity and position of substituents in the aromatic nucleus²³. As mentioned above, thiocyanate and cyanide are both detoxication products of malononitrile²⁴. The injection of 4-nitroBMN resulted in six to eight-fold rises in the level of blood thiocyanate of tumour-bearing human subjects¹⁰⁸. Cyanide has been found after intravenous administration of CS to dogs⁸⁸. In mice, symptoms of poisoning by injected malononitrile and CS are very similar in the short term (up to about 90 min), whereas the LD₅₀ values, expressed on a molar basis, are almost identical²³. Thiosulphate is effective in reversing the toxicity of cyanide¹²², of malononitrile^{18,21,23}, of BMN²³, and of CS^{23,88}. In the case of CS, reversal may be operative only in the short term²³. The results have been interpreted on the basis of conversion of the nitriles to free cyanide *in vivo*²³. Three other BMN derivatives (4-hydroxy, 3-nitro and 4-nitro) are also poisonous to mice, but, because the effects were insensitive to thiosulphate, cyanide liberation was not considered to be the cause of death²³.

Other workers⁸⁸ who have reported that thiosulphate protects against the poisonous effect of injected CS in rats have explained the role played by the anion in terms of direct chemical reaction with the compound rather than of the classic detoxicating vehicle for cyanide (for example, see refs. 24, 122). First-order rate constants were derived for the reaction of thiosulphate with CS (0.07) and with *o*-chloroacetophenone (0.02); on the basis of the similarity of these values, thiosulphate might be expected to afford at least some measure of protection against poisoning by *o*-chloroacetophenone, but this was not the case⁸⁸. The constants for the reactions between CS and phosphate (0.06) and CS and thiosulphate (0.07) are so close that

the actual process which occurred in both cases was probably hydrolysis. Phosphate buffer has been used in studies of the hydrolysis of BMNs, but actual participation of the anion was not reported⁷². When suspended in aqueous sodium thiosulphate, CS and four other BMNs failed to dissolve, even after several days at room temperature. In similar conditions the reaction between CS and sodium cyanide^{14,70} was largely complete in 15 min, and the solution was clear within an hour (G. R. N. J., unpublished observations). The fate of the aryl nucleus in the body has received very little attention; the principal detectable metabolite of CS in the rat is 2-chlorohippuric acid⁸⁸.

A few biochemical effects of BMNs have been studied *in vitro*. The powerful actions of 4-hydroxyBMN and 2-chloro-5-nitroBMN (5-nitroCS) in uncoupling oxidative phosphorylation have been described in both plant and animal systems¹²³. At a concentration of 1 mM, which is well above a tolerable level, CS partly inhibits lactate dehydrogenase. The reduced form of lipoic acid has been suggested as a more likely biochemical target for the agent⁸⁸.

There seem to be at least two, or perhaps even three, ways in which CS may kill. In mice, deaths which were caused within an hour of CS injection were attributed to the internal liberation of cyanide, but when this effect was overcome by thiosulphate, most moribund mice died on the second day²³. The reason for these later deaths is not known, but may involve a biochemical lesion¹²⁴ or a response to general irritation⁸⁸. If animals which had received lethal doses of airborne CS were to die shortly after exposure, cyanide involvement might be justifiably suspected; in fact, deaths do not generally occur until at least one day after exposure, and often even later^{88,118}. None the less, the risk from cyanide poisoning cannot be disregarded. Dogs exposed to lethal doses of CS aerosols die in respiratory distress⁸⁸, suggesting that the cumulative effect of CS on the alveolar surfaces may cause interference with the processes of gas exchange (compare refs. 125, 126).

Holmes has put forward an explanation of the mechanism of nervous stimulation caused by CS^{84,127}. According to this hypothesis, CS could react with strongly basic amino-groups⁴⁷, such as might be found in lysine residues, to give benzylamine adducts. Enzymically-catalysed oxidation would then produce imines or their tautomers, followed by breakdown to the $-\text{CH}(\text{CN})_2$ carbanion and 2-chlorobenzoylamino derivatives at the nerve endings. Such a hypothesis, however, might account for only a single brief stimulus, after which the benzoylated receptors might not be expected to remain sensitive to the agent, unless prompt hydrolysis were to occur. Another more likely scheme might involve simply the formation of addition compounds of CS with specific chemical groupings, perhaps thiols, at receptive sites. Holmes referred to unpublished work in which thiol groups instead of amines are implicated when skin receptors of the frog are stimulated⁸⁴. If the free energy change of the system were sufficiently low, the rapid reversibility of such reactions could explain the powerful sustained stimulation which is observed.

Offensive Use and Other Aspects

No BMNs other than CS seem to have been actually used for offensive purposes. The earliest references to this particular application describe the compound as a riot control agent²⁻⁴. The use of CS in this capacity has been assessed⁵, and comparative investigations of the effects of CS, ω -chloroacetophenone^{2,3}, 10-chloro-5,10-dihydrophenarsazine^{117,128} and bromobenzyl cyanide¹²¹ have been made.

Many devices have been invented for dispersing CS^{6,7,9,11,12}. Methods of dispersal include detonation of a quantity of explosive, or discharge by means of a propellant gas or liquid⁶. The principle of dissemination by burning a self-combustible composition which contains CS has been patented in several forms^{6,7,9,11,12}. Another device comprises several subunits, and bursts after a preset time. Each unit contains a self-combustible mixture and on ignition propels itself in random

directions by the emission of CS-laden gases through a small orifice¹¹. In Britain, only a few weapons are available to the Army¹³. In the United States the number of types exceeds twenty. These range in capacity from the XM58 grenade (20 g) to the XM28 bagged agent dispenser (300 kg)^{8,10}.

The incorporation of BMN into an insecticide has been patented¹²⁹. Tri- and pentachloroBMNs possess fungus controlling activity⁶², while 2,4-dichloroBMN is active against insects, nematodes, plants and fungi¹³⁰. Dialkyl and 4-acetoxyalkylBMNs are poisonous to a wide range of organisms, including mosquito larvae, nematodes and fungi⁶¹. Dialkyl thiophosphate derivatives of BMN are active against insects and fungi⁶³; the problem of which part of the molecule is primarily responsible for toxicity still remains.

The effect of solvents on the nuclear magnetic resonance of BMNs has been investigated¹³¹. Spectra and molar extinction coefficients have been published for a number of BMNs^{15,42,45,49,67,68} and for CS^{15,45,68,132}. Infrared absorption bands of BMNs, including CS, have been obtained¹³³, and CS has also been examined by gas-liquid chromatography (ref. 134 and G. R. N. J. *et al.*, to be published).

The radioprotective effects of certain BMNs have been studied¹³⁵. The incorporation of BMN, CS and 4-chloroBMN into rodent-repelling compositions has been patented¹³⁶ and this particular application of BMN has been evaluated¹³⁷.

Further unpublished work is mentioned in the final report¹³⁸ of the Himsworth Committee.

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Metabolic Coupling, Ionic Coupling and Cell Contacts

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Observations using electron microscopy and freeze-etching techniques show that both metabolic coupling and ionic coupling in fibroblasts are associated with the appearance of "gap" junctions.

THE concept of ionic communication arose from studies on excitable animal cells which indicated that there are low-resistance pathways between cells in contact which can facilitate the intercellular passage of ions¹⁻³. More recently, the passage of dyes and small molecules between excitable cells which are ionically coupled has also been observed^{4,5}. In excitable cells, ionic coupling is responsible for a functional electrical synapse or electrotonic junction. These occur where nerve or muscle cells must respond in rapid synchrony^{1,3}. Studies on cell contacts between coupled cells have suggested that the gap junction serves as the low-resistance pathway⁴. Low-resistance ionic coupling has also been described between cells in non-excitable animal tissues², but here the function of such coupling remains unclear. Because ionic coupling can exist between both normal and transformed animal cells in culture^{1,6,7}, cultured cell lines offer an appropriate system to study the function of ionic coupling in non-excitable cells.

In this study we have examined the relationship between two presumably independent contact phenomena in cell cultures—ionic coupling and the intercellular passage of factors involved in hypoxanthine metabolism, so-called "metabolic cooperation"⁸⁻¹¹. We have also looked for a possible ultrastructural basis for such intercellular communication between cells *in vitro*.

Metabolic Coupling

Cells of a permanent fibroblastic Chinese hamster line (Don) which have the capacity to incorporate the exogenously supplied purine ³H-hypoxanthine into their nucleic acids were grown in mixed culture with either of two different variant fibroblastic lines (DA and A9) which cannot incorporate the purine from the culture medium. Cells of the Don line were shown to contain the enzyme inosinic guanylic pyrophosphorylase (E.C. 2.4.2.8; IMP, GMP: pyrophosphoribosyl transferase) by autoradiography and by direct enzyme assay (unpublished results) and are termed IPP⁺ cells. By similar methods, both the DA and A9 cells, which cannot incorporate the exogenous purine, have been found to lack this enzyme activity and are therefore designated IPP⁻ cells. The DA cells are a clonal subline of the Don cells and are resistant to the

purine analogue 8-azaguanine at a level of 30 µg ml.⁻¹ in the growth medium (J. Graves, personal communication). The A9 cells are resistant to 3 µg ml.⁻¹ of 8-azaguanine and were originally derived as genetic variants of the mouse L cell line¹².

To distinguish accurately one cell type from the other in a mixed culture, the Don cell line was usually prelabelled by allowing incorporation of carbon or carmine particles into their cytoplasm¹³ or, alternatively, ³H-thymidine was used to prelabel the Don cell nuclei for autoradiography. The labelled Don cells were then mixed with unlabelled DA or A9 cells for study (Fig. 4, inset). Ionic coupling and ultrastructural examinations were made only on interacting cells (Don : DA and Don : A9) which could be suitably identified with the carmine labelling procedure.

All cell lines were grown in prescription bottles in Dulbecco's modification of Eagle's medium supplemented with 10% foetal calf serum and 5% lactalbumin hydrolysate and containing 100 µg ml.⁻¹ of both penicillin-G and dihydrostreptomycin.

At the beginning of an experiment appropriate label (carmine, carbon, or ³H-thymidine) was added to 3-day log phase cultures of Don (IPP⁺) cells 24 h before co-cultivation with unlabelled IPP⁻ cells (DA or A9) with a suspension of labelled Don cells at ratios ranging from 300 : 1 to 10 : 1 of IPP⁻ : IPP⁺ cells. Aliquots of these mixed populations were plated out at appropriate dilutions into 60 mm Falcon tissue culture dishes containing one or two 22-mm glass coverslips under 5 ml. of growth medium. After 24 h of co-cultivation at 37° C in a CO₂ incubator, ³H-hypoxanthine at a final concentration of 0.5 µCi ml.⁻¹ was added to the mixed cultures for periods ranging from 4–12 h. The coverslips were then fixed in ethanol : acetic acid (3 : 1), extracted for 30 min with cold 5% trichloroacetic acid, processed for autoradiography, and finally stained for 10 min with diluted Giemsa.

In mixed cultures of Don and DA cells (Don : DA) exposed to ³H-hypoxanthine, the Don cells always incorporated large amounts of the labelled purine into their nucleic acids whereas isolated DA cells, or groups of DA cells alone, rarely, if ever, had incorporated labelled purine above the level of background activity (Fig. 1). But DA cells, either directly or indirectly in contact with Don cells, did incorporate the isotope, though considerably less than the Don cells. This labelling pattern was always seen in this type of mixed culture no matter which technique was used to prelabel the Don cells. The gain of a metabolic function by the IPP⁻ DA cells appears to require direct cell-to-cell contact with the IPP⁺ Don cells and is similar to the results reported by others using the same metabolic markers in different fibroblastic cells^{8,9,13,14}.

An entirely different result was found when the Don (IPP⁺) cells were cultured with the A9 (IPP⁻) mouse cells (Don : A9). When labelled purine was added to these mixed cultures, the Don cells incorporated large amounts of label, but the A9 cells never incorporated the isotopic label above the level of background, even when they appeared to be in direct physical contact with the Don cells (Fig. 2).

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Ionic Coupling

Coverslips containing mixed populations of either Don : DA or Don : A9 cells were rapidly removed from the culture dishes and placed with original culture medium into a chamber with a bottom made from another larger coverslip. An additional layer of mineral oil was added above the culture medium to maintain original gas equilibrium, prevent drying out, and to minimize the effect of the meniscus on micropipette movements. The experimental chamber, placed on a fixed stage, was surrounded by a metal water jacket heated to maintain the medium at approximately 37° C. Cells were viewed with a $\times 40$ water immersion lens and an optical system comprised of a Leitz Labolux II body and a Reichart Nomarski Phase interference system. Zeiss micromanipulators were used to

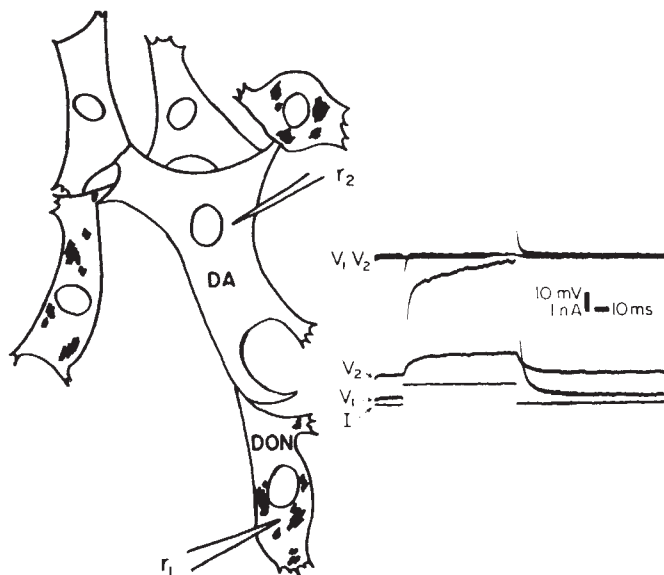


Fig. 3 Outlines of cells and micropipettes drawn from photomicrograph taken during a study of a mixed Don : DA culture. At right, tracings from the CRT are photographically superimposed. The upper trace (V_1 , V_2) indicates the zero potential and also the effect of passing current (I). The artefact in V_1 is barely detectable because of good bridge balance. After penetration of cells (resting potentials measured as initial vertical positions of traces V_1 and V_2) the same current produced a large depolarization of V_1 and a lesser depolarization of V_2 , indicating ionic coupling between Don and DA cells. Calibrations as shown.

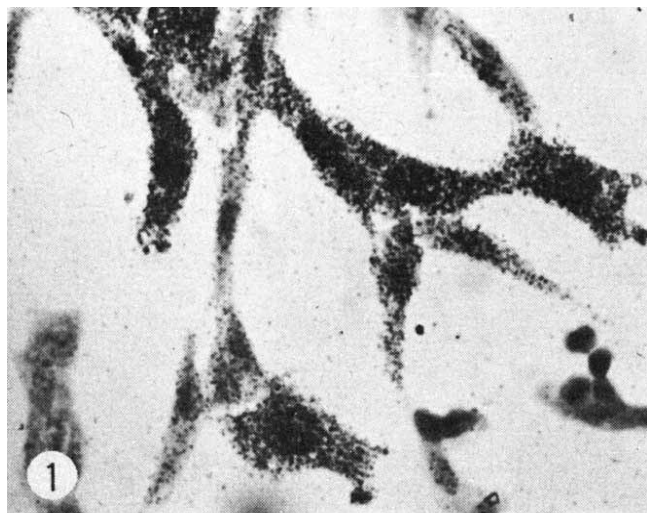


Fig. 1 Autoradiograph of a mixed culture of Don (IPP⁺) and DA (IPP⁻) cells. Nuclei of the Don cells were prelabelled with ^3H -thymidine ($0.1 \mu\text{Ci ml}^{-1}$ for 24 h) before mixing with unlabelled DA cells. After 24 h of co-cultivation, $0.5 \mu\text{Ci ml}^{-1}$ of ^3H -hypoxanthine was added for 4 h. Don cells have heavily labelled nuclei and cytoplasm. DA cells are unlabelled when isolated from Don cells (lower left) but are intermediately labelled when in contact with Don cells (vertical chain of DA cells left of centre) ($\times 630$).

Fig. 2 Autoradiograph of a culture of Don (IPP⁺) and A9 (IPP⁻) cells co-cultured for 24 h and exposed to $0.5 \mu\text{Ci ml}^{-1}$ of ^3H -hypoxanthine for 4 h. The Don cells have not been prelabelled with ^3H -thymidine in this experiment. The A9 cells are easily distinguished from the Don cells by their characteristic morphology (elongated spindle or stellate shape with a large oval, darkly staining nucleus). The A9 cells are never seen to contain label above the background level ($\times 480$). Inset: heavily labelled Don cell with a process lying over the process of an unlabelled A9 cell ($\times 600$).

position micropipettes. Suitable glass micropipettes filled with 4 M potassium acetate by standard technique invariably had a resistance between 100–200 M Ω . Only micropipettes with tip potentials less than -40 mV were used.

One micropipette (r_1) was connected through an AgCl/Ag electrode to the input probe of a WPI mk I preamplifier. The second micropipette (r_2) was connected through an AgCl/Ag electrode to the G_1 input of a Grass P18 differential high Z, negative C, preamplifier. Separate indifferent electrodes (agar saline/AgCl/Ag) for the two recording circuits minimized electrical artefacts. Just before impaling cells, measured direct current (d.c.) electrical potentials V_1 and V_2 were adjusted on the oscilloscope (CRT) screen to be the same (defined as system zero) for both r_1 and r_2 micropipette circuits. A square pulse of current (0.1–10 nA) was injected through micropipette r_1 and the direct effect on V_1 was compensated by the use of the "bridge" circuit of the WPI preamplifier. Following impalement of the cells, a negative inside "resting" potential of between -20 and -70 mV was recorded in most instances. Only penetrations where resting potentials in both cells initially exceeded -40 mV were used. The coupling coefficient (V_2/V_1) is used to express the amount of ionic coupling between two cells and was calculated as the steady state voltage change produced by the injected current measured in circuit r_2 divided by the steady state voltage deflexion produced simultaneously in circuit r_1 .

In mixed Don : DA cultures, Don cells labelled with carmine are ionically coupled to DA cells lacking carmine as well as to other Don cells with carmine (Fig. 3). In these cultures the DA cells are also coupled to other DA cells. The extent of coupling between adjacent cells may be as "close" as 0.5 (V_2/V_1). In two cases, however, it was found that artificially "close" coupling could be produced by damaging the cell impaled by the r_2 circuit. In general, as the distance between cells that are impaled increases (up to 50 μm in a chain of three cells) the coupling values may vary from less than 0.1 to 0.3; coupling was decremental with distance. In two cases, ionic coupling was studied between two cells interacting by long cell processes of 60 and 120 μm , respectively. In both cases, the cells were coupled, although not very closely.

In the Don : A9 cultures, the labelled Don cells are not ionically coupled to A9 cells (coupling less than 0.001 might not have been detected, but no coupling was observed in over ten trials). In the same cultures, Don cells labelled with carmine are coupled to other labelled Don cells, while A9 cells are not coupled to other A9 cells. The question arises as to whether coupling was mechanically destroyed in the Don : A9 combinations. In the same cultures, even damaged Don cells (with low resting potentials and low apparent membrane resistance) were coupled to each other; while, on the other hand, two Don cells with an A9 cell between them (Don : A9 : Don) were not coupled. We think that damage is unlikely to have caused uncoupling between the Don and A9 cells.

Cell Contacts

For electron microscopic studies, the Don (carmine labelled): DA and the Don (carmine labelled) : A9 cell populations were treated with (a) a lanthanum-glutaraldehyde fixative¹⁵, or (b) a cacodylate-buffered (0.1 M) 3% glutaraldehyde solution for 15–20 min. In both cases, the cells were post-fixed in cacodylate-buffered 1% osmium tetroxide, block-stained with uranyl acetate, and embedded in 'Epon 812' in the original plastic culture dishes in which they were grown. The embedded monolayers were studied with a phase contrast microscope and suitable areas for examination were selected and marked with a slide-marking objective. Selected areas were then cut out and mounted on prehardened blocks and serial sections of the cells were obtained by sectioning in a horizontal plane to the monolayer. For freeze-etching with a Balzers device¹⁶, the

mixed cell populations were fixed briefly with treatment (b) and then removed from the culture dish with a rubber policeman. Examination with a phase contrast microscope revealed that the contacts between cells had not been detectably altered by this procedure. Subsequently, the cells were gently pelleted in a centrifuge, treated with 20% glycerine for 2 h, and then frozen on cardboard disks for freeze-etching.

The ionic and metabolic information suggests that "light microscopic contact" is present and required between interacting Don : DA cells, so all detectable routes of cellular interaction between these cells were studied with serial sections and freeze-etching. Unlike normal fibroblasts studied in monolayer¹⁷, the Don : DA cells have a narrow (130 Å) intercellular space between them. No less than five general modes of cell interaction could be detected between these cells (Fig. 4): microvillar interactions, close associations of the cells (less than 130 Å), pinocytosis, possible focal tight junctions, and gap junctions¹⁵. Although no extensive tight junctions have been seen, structures resembling focal tight junctions occur frequently between the Don : DA cells. In some cases, these structures may actually be regions of very small or focal gap junctions. The gap junctions, although difficult to detect even with serial sections, were found in the Don : DA cells with both lanthanum impregnation and freeze-etch. The gap junction (Fig. 5) could be positively identified between the Don : Don, Don : DA, and DA : DA cells. In general, the average diameter of the plaque-like gap junctions between these cells was only 0.3 µm. With lanthanum impregnation, a 30–40 Å gap was resolved between the membranes and the characteristic polygonally packed 85 Å membrane particles were present with

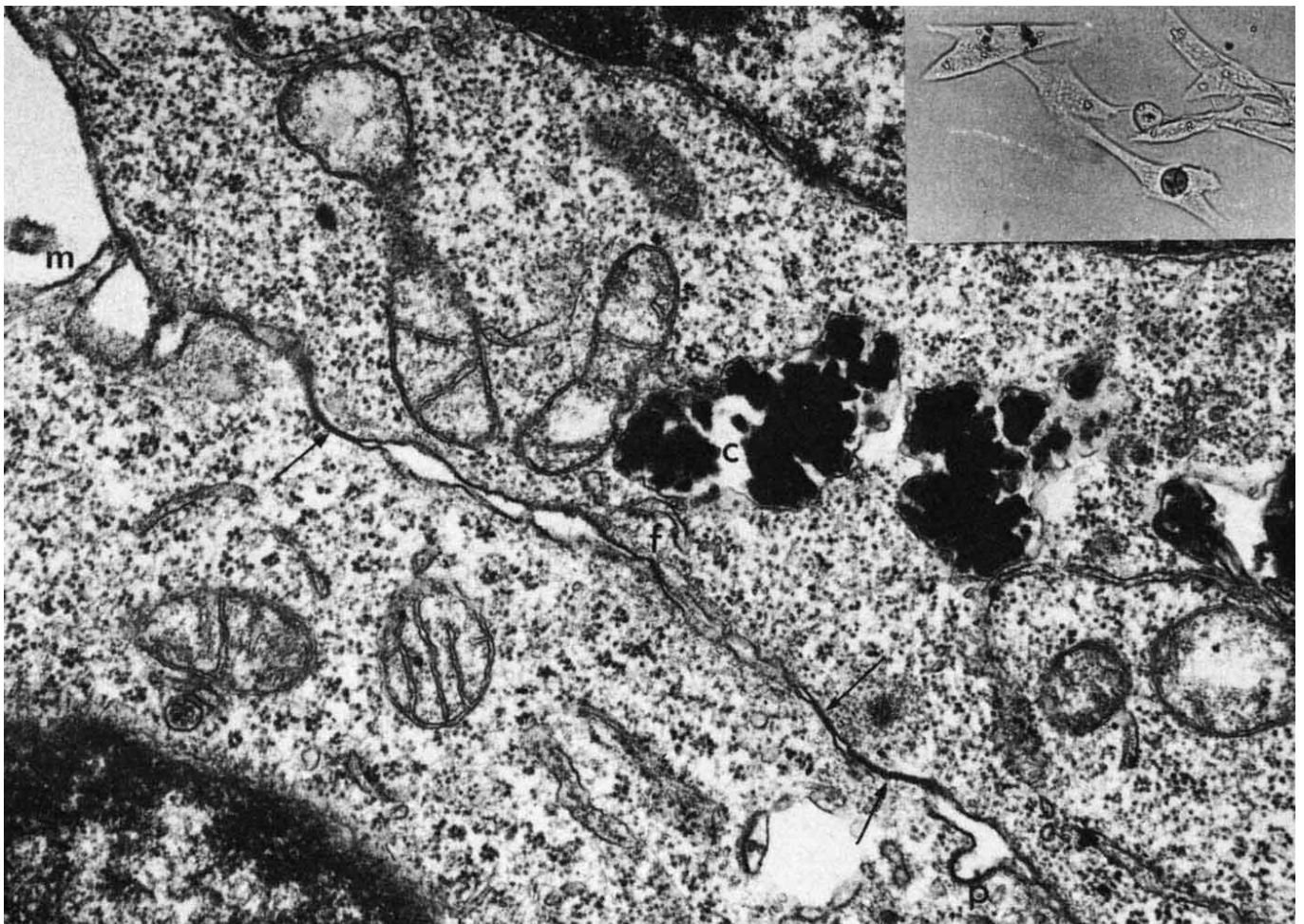


Fig. 4 Cell interactions between a carmine (c) labelled Don cell and an unlabelled DA cell. Three gap junctions between the cells are indicated with arrows. A microvillus (m), a pinocytotic invagination (p), and an apparent focal contact (f) are also present ($\times 36,000$). Inset: phase contrast picture of a carmine labelled Don cell contacting a chain of unlabelled DA cells ($\times 600$).

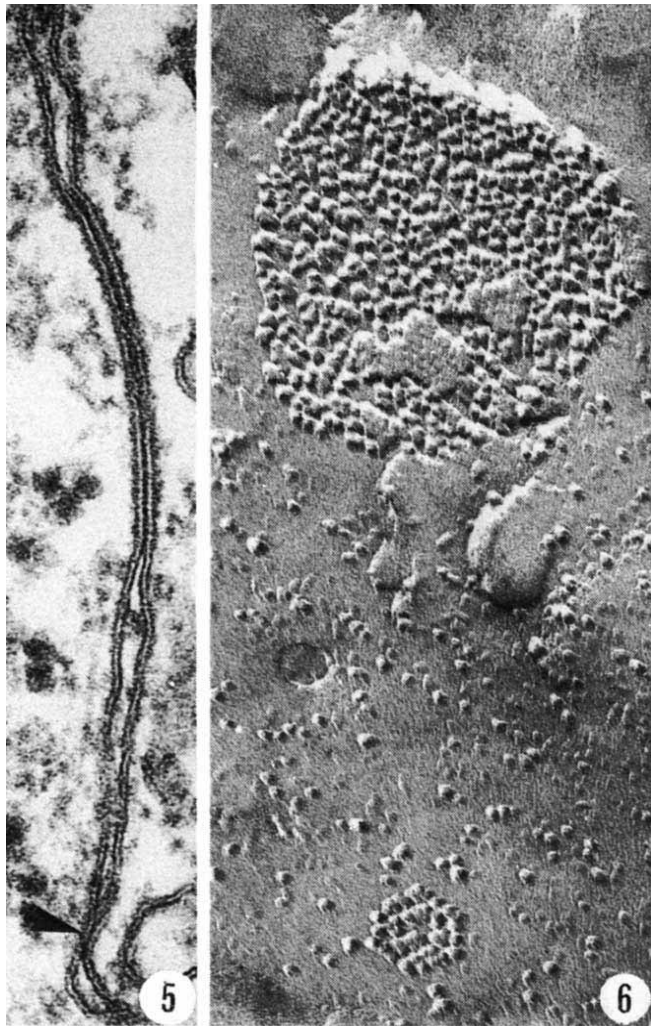


Fig. 5 Thin section image of gap junction between two DA cells in the Don : DA mixed cell culture. The two cell membranes approximate each other to form a junction with a 35 Å "gap". Below (arrow) a focal tight or focal gap junction is also present; but it is not possible to identify this structure accurately on the basis of this image alone ($\times 180,000$).

Fig. 6 Freeze-etch image of gap junctional membrane differentiation obtained in Don : DA mixed culture. In the fracture face, a large plaque-like aggregate of the 85 Å membrane particles is present, as well as a smaller, polygonally packed junctional aggregate ($\times 180,000$).

freeze-etch (Fig. 6). With both techniques, the gap junction closely resembled others that have been described in animal tissues^{4,5,18,19} and in cell cultures^{17,20}. Besides the larger gap junctional particle plaques, smaller particle aggregates were also observed which might indicate the transient nature of the gap junction between these cells.

Examination with the electron microscope showed that the Don : A9 cultures also possessed all the types of cell interactions present in the Don : DA cultures, including the gap junction. In the Don : A9 cell populations, however, we have been unable to identify gap junctions between either Don : A9 cells or A9 : A9 although all other types of interactions have been observed between these cells (Table 1).

We have found that Don cells, which influence the metabolic capacity of DA cells, are ionically coupled to, and make gap junctions with, DA cells. The metabolic capacity of A9 cells is not influenced by Don cells even though they appear to contact, and we have been unable to find either ionic coupling or gap junctions between Don and A9 cells. These results strongly suggest that the passage of ions^{1,7} and certain metabolically important molecules^{9,21} between fibroblastic cells in culture occurs simultaneously and may be dependent on

Table 1 Summary of Results on Don : DA and Don : A9 Cell Populations

Cells	Meta- bolic coup- ling	Ionic coup- ling	Gap junc- tion	Pino- cyto- sis	Focal tight junc- tion	Close asso- cia- tion	Micro- villi
In Don : DA							
Don : Don		*	*	*	*	*	*
Don : DA	*	*	*	*	*	*	*
DA : DA		*	*	*	*	*	*
In Don : A9							
Don : Don		*	*	*	*	*	*
Don : A9	—	—	—	*	*	*	*
A9 : A9		—	—	*	*	*	*

the presence of a specific cellular structure, the gap junction. These observations, however, do not clarify whether the gap junction serves as the only structural pathway for metabolic and ionic coupling, or even whether these two contact phenomena must share the same pathway.

The absence of ionic and metabolic coupling, along with gap junctions, between closely contacting Don : A9 or A9 : A9 cells might result from a genetic deficiency in the A9 cells which prevents them from making such functional contacts. Recent observations indicate that the A9 cells cannot metabolically couple with either wild-type (IPP⁺) L cells or wild type BHK cells; further, wild type L cells do not interact with (IPP⁻) BHK cells²². Another report indicates that certain L cells are also incapable of ionically interacting with myocardial cells²³. The coupling failure may also be the result of a non-permissive culture environment for these A9 cells. Culture conditions have been reported to have decisive effects on both ionic^{1,7} and metabolic⁹ coupling. Regardless of the reasons for the failure of the A9 cells to communicate with contacting Don cells, the absence of both ionic and metabolic coupling, as well as the apparent absence of gap junctions, is interesting considering the proposed role of gap junctions and ionic coupling *in vivo*^{1-5,15}.

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Galapagos Gore, NazCoPac Triple Junction and Carnegie/Cocos Ridges

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In this article a solution is offered to the problem of the Galapagos "gore", a triangular region in the equatorial eastern Pacific which includes within its boundaries the Galapagos Islands.

THE Galapagos gore (Fig. 1) has its apex at the intersection of the Nazca, Cocos and Pacific rigid lithospheric plates, called in this article the NazCoPac triple junction. Extending eastward from this apex, the gore is apparent as a region of anomalous structure and topography which is excel-

lently displayed, for example, on the new Scripps Institution of Oceanography bathymetric charts of the North Pacific¹. The base of the gore is defined by the Panama fracture zone (a transform fault), which begins at the Middle America trench and then forms the northern half of the base of the gore triangle. The southern half of the base is formed by its extension as a seismically dead fracture zone.

The gore has an apical angle of 36° which is asymmetrically bisected by the Galapagos rift. These rift zones have only been partially surveyed, but parallel magnetic anomaly patterns have been identified back to anomaly 3 on the scale of Heirtzler *et al.*², indicating active seafloor spreading and emplacement of new oceanic crust. The higher ground of the gore as compared with the surrounding Pacific we infer as due to slow spreading within the gore (which produces high, rugged

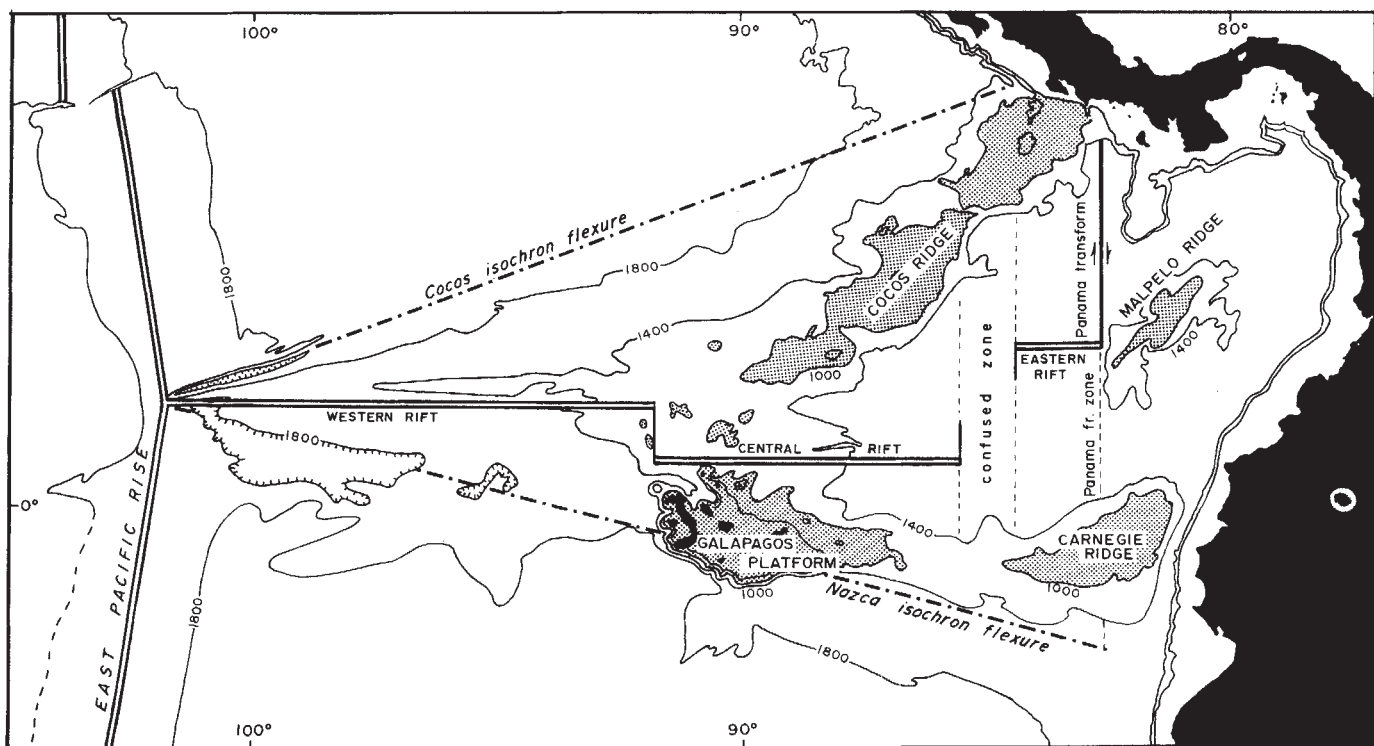


Fig. 1 Bathymetry of the Galapagos gore with prominent topography above 1,000 fm shaded. The gore, delimited by the Carnegie and Cocos isochron flexures, is defined by high relief and linear basins near the NazCoPac triple junction. Modified from Chase *et al.*¹ and Chase¹⁶.

topography) and fast spreading on the limbs of the Pacific Rise rift (which produces a low, smooth rise). The overall dimensions of the gore are: length of wings, 2,200 km; length of base, 1,200 km; total area, 1.3×10^6 km².

The gore encloses two large ridges which bifurcate from the Galapagos Islands. These are the Cocos Ridge and the Carnegie Ridge (the latter may be actually construed to include the Galapagos Islands). We believe that these ridges form a binematath—that is, two thread ridges of mostly alkaline basalt which have been laid down as the moving lithospheric crust drifted over a fixed, or almost fixed, hot spot—the Galapagos hot spot. This involves the belief that a plume of magma is rising from the deep upper mantle (175 km or more), or below the zone of shear of the moving crustal plates. As this region seems not to be in motion, the resultant nematath spilled on the ocean floor provides an absolute measure of the drift of a crustal plate. In addition, we infer that the central Galapagos rift has remained near the hot spot so that lava extruded has been alternately spilled on both the Cocos and Nazca plates. We have already suggested that the Walvis and Rio Grande ridges emanating from the Tristan da Cunha hot spot are another example of a binematath³.

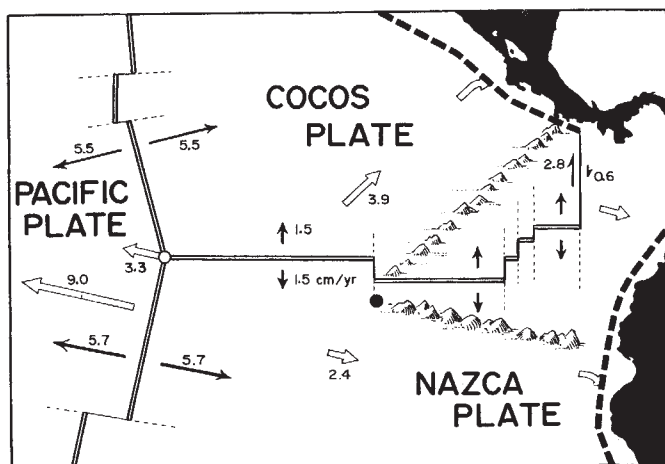


Fig. 2 Schematic representation of the Cocos and Carnegie ridges as nemataths emanating from the Galapagos fixed hot spot near the Cocos and Nazca plate boundary. Open arrows indicate absolute magnitudes and directions of plates and NazCoPac triple junction in cm/yr. Solid arrows indicate relative magnitudes and directions of plate boundaries.

Although the map of Chase *et al.*¹ referred to the Galapagos rift as a fracture zone, seafloor surveys by Raff⁴, Herron and Heirtzler⁵ and Grim⁶ clearly show it to be a region of rifting and seafloor spreading. In fact, three rifts have been mapped which we can term the Western Galapagos Rift, the Central Galapagos Rift, and the Eastern Galapagos Rift. These rifts are separated by transform faults, with the area between the central and eastern rifts being a zone of confusion (a stepladder of faults and rifts).

Relative and Absolute Plate Motions

The relative and absolute plate motions are presented in Fig. 2.

The spreading rate at the NazCoPac triple junction is not known from magnetic surveys. But this rate should be about 5.7 cm/yr based on a rate of 6.0 cm/yr reported 20° further south along the same rift², which is at essentially the equator for the Pacific/Nazca relative pole of rotation, at latitude 53° N, longitude 47° W (south of Greenland)⁷. Under the premise of rigid plate rotation, the spreading rate varies as the cosine of the latitude with respect to the pole of rotation. Geometrical considerations then require the spreading rate to be 5.5 cm/yr to the north of the triple junction. There is no

need to infer a zone of compression in the Pacific plate to the west of the triple junction as suggested by Raff⁴ and by Herron and Heirtzler⁵ which would be in violation of rigid plate tectonics. At triple junctions, seafloor spreading cannot be perpendicular to all three of the rifts. Two or possibly three of the rifts must insert dikes of new crust oblique both to the rifts and to the magnetic anomaly patterns. This avoids compression.

By using these spreading rates, we have erected 10 m.y. isochrons (lines of equal age of oceanic basement) for the seafloor outside the gore (Fig. 3). The seafloor thus grows progressively older to the east, finally attaining an age of 40 m.y. at the zone of subduction. Seafloor spreading theory requires that these isochrons be parallel to rifts. So within the gore we have erected isochrons parallel to the Galapagos rifts. Their spacing is fixed by the apical angle of the gore, because these interior isochrons must abut the exterior isochrons. The isochrons thus turn through an angle of 75° at the gore margin and their trend creates an isochron flexure. This geometry imposes a seafloor spreading rate within the gore of about 1.6 cm/yr (or a plate separation rate of 3.2 cm/yr). It should be noted that our rate of seafloor spreading is less than that inferred from earlier surveys^{4,6}; for example, Heinrichs (in Van Andel *et al.*⁸) infers a spreading rate of 2.5–2.8 cm/yr for the eastern rift. But these surveys go back only to anomaly 3 (5 m.y. BP). Accepting such a high spreading rate would be to increase considerably the apical angle of the Galapagos gore, which would violate the bathymetric data.

Using the nematath concept of fixed hot spots, we can determine the absolute vectors for the Pacific, Nazca and Cocos plates in the Galapagos region. The Hawaiian nematath increases in age from zero at Hawaii to 26 m.y. BP at Midway⁹ and to 40 m.y. BP at the junction with the Emperor Seamounts¹⁰. This gives the Pacific plate along the trend of the Hawaiian Chain a velocity of 9 cm/yr. This same velocity applies for the Pacific plate at the NazCoPac triple junction, because it lies along the same line of latitude relative to Morgan's¹⁰ absolute pole of rotation for the Pacific plate at latitude 67° N, longitude 73° W. The East Pacific rift is opening at a rate of 11.4 cm/yr, so the NazCoPac junction must be moving north-west at 2.4 cm/yr bearing 277°. This is also the bearing of the isochron flexure within the Nazca plate, suggesting that the triple point has migrated along this flexure in the past. By similar vector subtraction the Nazca plate appears to have an absolute drift of 2.4 cm/yr bearing 095° (based on trend of the Carnegie ridge), or essentially in the opposite direction; and the Cocos plate a drift of 3.3 cm/yr bearing 045°.

The absolute drift rate of the Pacific plate of 9 cm/yr at the Galapagos triple junction is critical to our argument for the age of the Galapagos gore. For example, an increase of the spreading rate to 7.0 cm/yr (instead of 5.7 cm/yr) along the East Pacific Rise would then give a separation rate (twice the spreading rate) of 14 cm/yr. Subtracting 9 cm/yr (the absolute drift rate of the Pacific plate) from the 14 cm/yr separation rate with respect to the East Pacific rift results in a 5 cm/yr absolute drift rate for the Nazca plate. This is a doubling of the velocity of the Nazca plate which in turn would make the Galapagos gore only 20 m.y. old instead of 40 m.y. old. Thus, a higher rate of spreading along the East Pacific Rise profoundly affects our seafloor isochrons and timing. The principles involved in our interpretation would, however, remain valid.

The Galapagos rifts within the gore do not exactly bisect the gore as they should if seafloor spreading were strictly symmetrical, with the same amount of seafloor being added on both limbs. But the western and eastern Galapagos rifts are essentially central, and it appears that there has been only slight asymmetry in spreading, with greater spreading to the north than to the south. In contrast, the Central Galapagos rift requires highly asymmetrical seafloor spreading or, alternatively, the rift taking successive small jumps to re-position itself southward. The Galapagos hot spot probably imposes a weakness on the lithosphere, which tends to keep the rift

over the hot spot. To show the effect of such a jump on the seafloor isochrons, we have arbitrarily assumed a jump at 10 m.y. BP of the central rift (Fig. 3). The tendency for a hot spot to force the relocation of a spreading rift can also be observed at Iceland. This hot spot apparently causes a 175 km eastward offset of the Mid-Atlantic rift relative to an extrapolated trend connecting the Reykjanes rift directly to the Jan Mayen rift.

Five "basement ages" in the region have been reported by the Deep Sea Drilling Project (JOIDES)^{11,12}. These are: for leg 9, cores No. 83 and 84; and for leg 16, cores No. 155, 157 and 158. Ages of the lowermost sediments from these cores are: 11 m.y. BP at core 83; 10 m.y. BP at core 84; 15–20 m.y. BP at core 155; 10 m.y. BP at core 157; 15–20 m.y. BP at core 158. Although core 83 agrees rather closely with our isochron, all "basement dates" are somewhat younger than the basement indicated on our isochron map (Fig. 3). These data possibly

Cocos nemataths are appropriately reduced in length and they both mark the absolute drift vectors of the plates on which they are imposed. Since the Nazca plate is moving parallel to the southern isochron flexure, the opening of the gore is due entirely to the drift of the Cocos plate along a bearing of 045°. The Galapagos rifts migrate northward attempting to maintain a central position with the gore. This is not achieved by the Central Galapagos rift because it tends to hug the hot spot.

Fig. 5 depicts the gore 30 m.y. BP, or 10 m.y. after its birth. The gore was then very small, and the triple junction was near the hot spot. A long transform fault connected the new rift to the Middle America subduction zone (off the diagram). In this construction, the close fit of the Carnegie and Cocos ridges along their 1,000-fm isobath is intriguing. Even earlier (40 m.y. BP), the hot spot and the triple junction would be joined and the gore would vanish to zero. This would mark

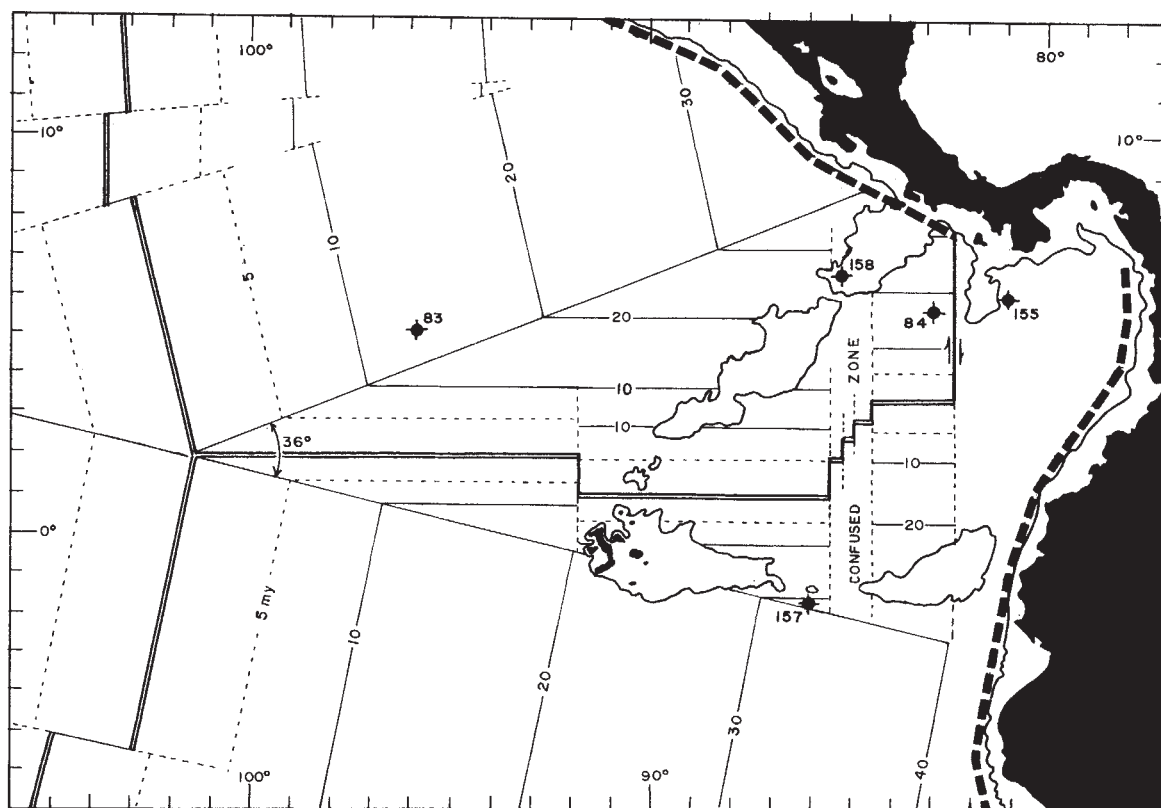


Fig. 3 Isochron map based on seafloor spreading. Glomar Challenger stations at which basement ages are available or inferred denoted by spiked circles. Mid part of Galapagos rift isochrons constructed to show a southward rift jump of about one degree 10 m.y. BP.

suggest that the Galapagos gore is younger than we have surmised. Considering the uncertainties involved in the JOIDES dates (later effusions of lava onto the seafloor; lava from nemataths which were emplaced later than the basalt injected into spreading rifts and so on), we believe that our construction is constrained only to the extent that our seafloor age dates always must be older (and they are) than the JOIDES dates.

Evolution of the Galapagos Gore

Fig. 4 shows the gore at 10 m.y. BP. This is obtained by projecting the NazCoPac triple junction backwards along the Nazca isochron flexure (a reversal of its modern migration vector) while holding the Galapagos hot spot fixed. We have assumed constant spreading and drift rates. The gore consequently shrinks in all dimensions. Both the Carnegie and

the birth of the gore, the triple junction, and the hot spot. Presumably the hot spot came first and initiated the new plate boundaries.

We can rule out the possibility of any large change in the angular geometry of the triple junction, a stable type of triple junction¹³. Such an effect would be to flare or constrict the isochron flexure, which is not observed. The Galapagos gore instead has straight wings, approximating an isosceles triangle.

We can also demonstrate that the triple point has migrated. If this were not so, the strike of the Cocos ridge as a nematath would be parallel to the trend of the isochron flexure marking the north limb of the gore (Fig. 3). The isochron flexure trend is actually less steep than the Cocos ridge, which can only mean that the triple point has migrated toward the west. In addition, the fact that the Cocos ridge strikes into the northeast corner of the gore demonstrates that the triple point and the hot spot were once at the same position when this far end

of the Cocos ridge was extruded. We offer the solution, therefore, that the angular geometry of the triple point has remained geometrically stable while it has migrated north-westward.

If it were possible to show that the Cocos ridge, in its entirety, is constrained within the limits of the gore, this would mean that the triple point was born at the Galapagos hot spot 40 m.y. BP. We suppose that this is probably true, but it cannot be demonstrated because an unknown amount of north-westward extension of the Cocos ridge may have been subducted into the Middle America trench and thus not exist today. The Malpelo ridge just to the east of the Panama fracture zone might possibly be such a remnant of the Cocos ridge. Such an interpretation would necessitate an earlier extension of the Galapagos rift zone into that region that had become inactive, leaving this part of the ridge frozen on the Nazca plate. If this were the case then the early gore would have extended further east of the hot spot than west. The hot spot would then predate the triple junction. On the other hand, if the hot spot existed prior to the creation of the triple junction, an ancient and now isolated nematath should be present on the Pacific plate, on a bearing of 277° from the triple point and commencing where the Pacific is older than 40 m.y. We can find no evidence for such a nematath. This suggests that the hot spot and the triple point were born at the same time.

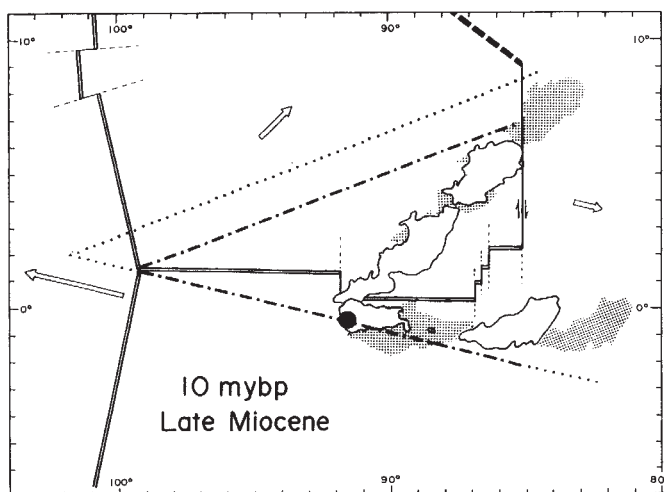


Fig. 4 Development of Galapagos gore 10 m.y. BP. Dash-dot line represents Cocos and Nazca isochron flexures at that time; dotted line represents the Recent position of the flexures. Recent positions of Cocos and Carnegie ridges shaded. Arrows as in Fig. 2.

Models Compared

Van Andel *et al.*⁸ have offered an alternative explanation for a portion of the Galapagos gore. They suggest that the Cocos and Carnegie ridges were once juxtaposed and then were split apart by the insertion of spreading rifts associated with a jumping transform fault. This infers that these two ridges are old (in that they pre-existed the creation of the Galapagos rift as the Nazca-Cocos plate boundary) and that the ridges are of equal age along their strike. Clearly, the volcanically active Galapagos Islands are youthful and they appear to grow older to the east¹⁴, a trend which we suppose can be extrapolated along the Carnegie ridge. It also implies that fast spreading occurred to the east and that this spreading decreased essentially to zero at the Galapagos where the two ridges converge. We question this model because it seems to violate some of the basic rules of plate tectonics. Their scheme would seem plausible if the triple point was fixed at the hot spot, but this is not the case.

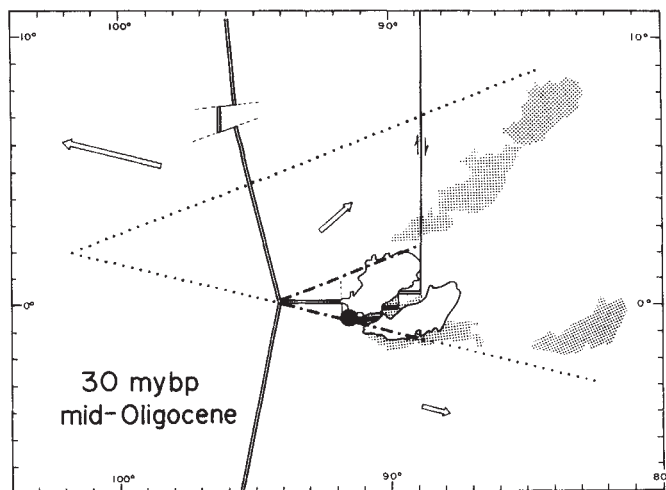


Fig. 5 Development of Galapagos gore 30 m.y. BP. See Fig. 4 for explanations.

The Galapagos Islands contain many endemic birds and bizarre animals which have required millions of years for their evolution in isolation. By our model, the modern Galapagos Islands may have inherited faunas from a whole series of ancestral "Galapagos islands" which existed over a span of 40 m.y. Presumably the animals would have little difficulty negotiating the short span of water to a new volcanic island as an older extinct volcanic island drifted eastward and subsided beneath the sea (a subsiding "stepping stone"), adding itself to the end of the Cocos and Carnegie ridges. To date, no guyots have been reported from either the Carnegie or Cocos chains, but this still is not conclusive evidence that these ridges were not subareal at some time in their history¹⁵.

In summary, we conclude the following. (1) The gore and the creation of the Galapagos rift (which in turn created the Cocos plate) is about 40 m.y. old; but possibly much younger if relative spreading rates along either the Galapagos or East Pacific Rise can be shown to be faster than those inferred here. (2) The NazCoPac RRR triple junction has maintained a stable angular configuration but has migrated away from the Galapagos hot spot along a bearing of 277° and a velocity of 3.3 cm/yr since its inception. (3) The Cocos and Nazca ridges form a binematath emanating from the fixed Galapagos hot spot, the bearing of each indicating the absolute vector of the two plates they surmount. (4) The opening of the gore, as a zone of extension, is new ocean floor resulting from the absolute drift motion of the Cocos plate to the north-east.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Radio Stars Beta Persei and Beta Lyrae

FOLLOWING the discovery that the radio emission of the Antares system originates from the B3 V component^{1,2}, observations of other binary star systems were undertaken with the NRAO interferometer operating at 2,695 and 8,085 MHz. Here we report the detection of radio emission from two well known eclipsing binaries, β Persei (Algol) and β Lyrae.

The observations were made from October 23 to November 10, 1971, on interferometer spacings of 900, 1,800 and 2,700 m. The methods of analysis have been described elsewhere¹. The positions of the point radio sources associated with the two stars are shown in Table 1, together with the optical positions derived from the FK4 catalogue. The positions are all for the date 1950.0, but proper motion corrections have been applied to the optical positions to correspond to the epoch 1972, in order to make them more closely comparable with the radio measurements. The mean errors of the optical positions include an allowance for the uncertainty in the proper motions. The radio position for the β Lyrae radio source was obtained from an aperture synthesis map of the field. Better hour angle distribution for the observations of β Persei permitted its position to be determined accurately by an absolute method³. The errors given with the latter position are mean errors.

Table 1 Radio and Optical Positions

Star	Radio position (1950.0)	Optical position (1950.0)
β Persei	$\alpha = 03^{\text{h}} 04^{\text{m}} 54.39 \pm 0.03 \text{ s}$ $\delta = +40^{\circ} 45' 52.0'' \pm 0.4''$	$\alpha = 03^{\text{h}} 04^{\text{m}} 54.363 \pm 0.003 \text{ s}$ $\delta = +40^{\circ} 45' 52.51'' \pm 0.04''$
β Lyrae	$\alpha = 18^{\text{h}} 48^{\text{m}} 14.0 \pm 0.2 \text{ s}$ $\delta = +33^{\circ} 18' 13.0'' \pm 3''$	$\alpha = 18^{\text{h}} 48^{\text{m}} 13.936 \pm 0.003 \text{ s}$ $\delta = +33^{\circ} 18' 12.47'' \pm 0.04''$

The close coincidence of the radio and optical positions leaves no doubt that the radio sources are associated with the stars. The data presented in Table 2 (dates and times of observation, with flux densities measured at the two frequencies) show further that the radio emission of β Persei is strongly variable at 2,695 MHz. The variation is less clearly established at 8,085 MHz. It is not yet certain whether β Lyrae is variable at these frequencies. Because the angular size of the radio emitting region in β Persei may be very small, it is possible the variation could be due primarily to scintillations imposed by the interstellar medium.

The spectra implied by the flux densities in Table 2 are not inconsistent with thermal radiation by an optically thick source. Nevertheless, the radiation is unlikely to be thermal. If the emission arises from an optically thick cloud of ionized gas at a temperature of 10^4 K, then the typical flux density of $1.5 \times 10^{-28} \text{ W m}^{-2} \text{ Hz}^{-1}$ observed at 8,085 MHz corresponds to an angular diameter of 0.2 arc s for the source. Taking the distances of β Persei and β Lyrae as 25 and 500 pc, respectively^{4,5}, we find corresponding linear diameters of 5 and 100 a.u. These values are many times larger than the separa-

tions of the binary pairs. This model would imply that the optical spectra of both stars should show strong nebular emission lines at all times, and the spectrum should show little, if any, variability in intensity or variable Doppler shifts related to the orbital motions of the embedded stars. This is quite unlike what is actually observed. The spectrum of β Persei only occasionally shows faint, variable H α and H β emission lines^{6,7} between eclipses, and they disappear during both the primary and secondary eclipses. Variable emission lines of He I 10830 are also seen in this system⁸. We thus conclude that the lines originate in a relatively small volume and that they are very transient features. In the case of β Lyrae, emission lines are usually present, but they vary in radial velocity in accordance with variations in the orbital positions of the stars.

Table 2 Times of Observation and Flux Densities

Star	Date	UT	Flux density ($10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$)	
			2,695 MHz	8,085 MHz
β Persei	Oct. 23, 1971	0543-1106	0.006 ± 0.004	0.015 ± 0.005
	Oct. 31	0445-0930	0.014 ± 0.003	0.019 ± 0.003
	Nov. 6	0026-1044	< 0.004	0.010 ± 0.003
	Nov. 7	0247-1041	0.011 ± 0.003	0.012 ± 0.003
	Nov. 8	0244-1037	< 0.005	0.015 ± 0.003
	Nov. 9	0239-0459	0.012 ± 0.005	0.006 ± 0.005
	Nov. 10	0235-0756	< 0.005	0.010 ± 0.003
β Lyrae	Nov. 4-5,			
	1971	2240-0241	0.006 ± 0.004	0.015 ± 0.004
	Nov. 5-6	2239-0011	< 0.005	0.020 ± 0.005
	Nov. 7	0018-0232	< 0.005	0.015 ± 0.005
	Nov. 7-8	1547-1645 2352-0228	< 0.005	0.011 ± 0.004
	Nov. 8-9	2343-0224	< 0.005	0.013 ± 0.004
	Nov. 9-10	2125-0220	< 0.005	0.015 ± 0.005

When the available data for β Persei are phased with the known orbital period of 2.8673 days, there is no clear indication of any correlation between the radio emission and the optical light curve. None of these observations, however, were made during a primary eclipse.

It should be noted that the four presently known radio stars, where the dominant star is optically "normal", have three characteristics in common. Antares B (refs. 1, 2), Cygnus X-1 (ref. 9), β Persei, and β Lyrae all involve a binary system; a B star; and clear evidence of gas streaming or moving in the binary system. These characteristics give what may be very important clues for future observations of other systems. When the common characteristics are more clearly established by future observations, they will undoubtedly assist in the theoretical interpretation of the interesting physical processes that must be going on in such systems.

Finally, it has recently^{10,11} been argued that certain peculiarities of β Lyrae could be explained by the idea that a black hole is present in the system. Whether this concept is applicable or not, the presence of probably non-thermal radio sources in β Persei and β Lyrae indicates that some unusual high energy processes may be occurring in these systems.

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Position and Identification of the Cygnus X-1 Radio Source

THE initial measurements^{1,2} of the position of the radio source associated with the X-ray source Cygnus X-1 were uncertain by 3 to 5 arc s. The results were consistent with two possible identifications with stars³: the 8.9 mag. BOIb star HDE 226868 (= BD + 34° 3815), and a 15 mag. red star 9 arc s to the north-west of the B star. Because of the apparent normality of the B star and possible signs of peculiarity in the red star³, it was not clear which should be identified with the radio source. Here, we report the resolution of the ambiguity by an accurate determination of the radio position.

The observations were made at frequencies of 2,695 and 8,085 MHz with the NRAO three-element interferometer at Green Bank from October 28 to October 31, 1971. The baseline lengths were 900, 1,800 and 2,700 m. Cygnus X-1 was observed concurrently with sixty other radio sources in a programme of precise position measurements. The method has been described previously⁴; certain refinements, however, were introduced in the analysis to eliminate the principal sources of systematic error. The procedures and results for the sixty sources will be described in detail later.

The position of the Cygnus X-1 radio source, with its standard errors, is found to be:

$$\alpha_{1950} = 19 \text{ h } 56 \text{ m } 28.87 \text{ s} \pm 0.02 \text{ s} \\ \delta_{1950} = +35^\circ 03' 55.0'' \pm 0.3''$$

The AGK2 position of HDE 226868 is

$$\alpha_{1950} = 19 \text{ h } 56 \text{ m } 28.88 \text{ s} \\ \delta_{1950} = +35^\circ 03' 54.9''$$

The nearly perfect positional coincidence of the radio source and the star leaves no doubt that HDE 226868 is the correct identification.

During the three-day observing period, the flux density for Cygnus X-1 was $(12 \pm 3) \times 10^{-29} \text{ W m}^{-2} \text{ Hz}^{-1}$ at 2,695 MHz and $(15 \pm 3) \times 10^{-29} \text{ W m}^{-2} \text{ Hz}^{-1}$ at 8,085 MHz.

It is now known^{5,6} that HDE 226868 is a spectroscopic binary with a period of 5.6 days. Bolton⁶ has found variable emission lines resembling those of P Cygni in high-dispersion spectra of this star. This strongly supports the identification of the radio and X-ray sources with the HDE 226868 binary system.

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Identification of Cygnus X-1 with HDE 226868

THE ninth magnitude BOIb star HDE 226868 is closely coincident with the position of Cygnus X-1 and its associated variable radio source¹. Dolan² has pointed out that Cyg X-1 appears to be a two component X-ray source. One component has a synchrotron spectrum, and the other has a thermal (or bremsstrahlung) spectrum. The latter component varies on a time scale of days in a way that suggests it is being eclipsed. Therefore I decided to take photographic spectra of HDE 226868 to look for velocity and spectrum variations that might be correlated with the X-ray fluctuations. I find that the velocity of the star varies with a period consistent with that of the X-ray variation. It may not be possible, however, to interpret the X-ray variations in terms of simple eclipses.

The spectrograms were taken between mid-September and mid-November 1971 with the 74-inch telescope of the David Dunlap Observatory in Richmond Hill, Ontario. All except one were taken at a dispersion of 12 Å mm^{-1} , but because of the star's faintness and unfavourable weather conditions it was necessary to use projected slit widths of 40 to 50 μm . These observations have been supplemented by results from 5 to 40 Å mm^{-1} plates taken in one night with the 90-inch reflector of the Steward Observatory of the University of Arizona. These plates were obtained and measured by Dr Roberta Humphreys, who communicated the results to us before publication. She also provided some additional observations of emission features in the spectrum, and Mr David L. DuPuy of the David Dunlap Observatory obtained UBV photometry of HDE 226868 on three nights.

The results of the radial velocity measures are given in Table 1 and the velocity curve is plotted in Fig. 1 for a period

Table 1 Radial Velocity Measurements

Julian date	V_r (km s ⁻¹)	Standard deviation (km s ⁻¹)	Inter- stellar K-line (km s ⁻¹)	H β emission (km s ⁻¹)	Disper- sion (Å mm ⁻¹)
*2441210.660	+15	± 9	-19.5		43
1213.665	-24	± 9	-15.7	+176	12
†1217.588	-58	± 10	-14.3	+39	12
			+74.7		
1224.581	-41	± 7	-10.7	+154	12
1225.696	+33	± 9	-15.6		12
1237.579	+57	± 15	-28.3		12
1245.555	-48	± 14	-12.6		12
1252.549	-62	± 9	-15.6		12
1259.524	+56	± 7	-14.4		12
1261.573	-15	± 8	-17.2		12
‡1263.7	-65	± 6			40

* Unidentified emission feature at $\lambda 4519.10 \text{ Å}$.

† Uncertain identification of H β emission.

‡ Mean of five plates taken by Dr Roberta Humphreys.

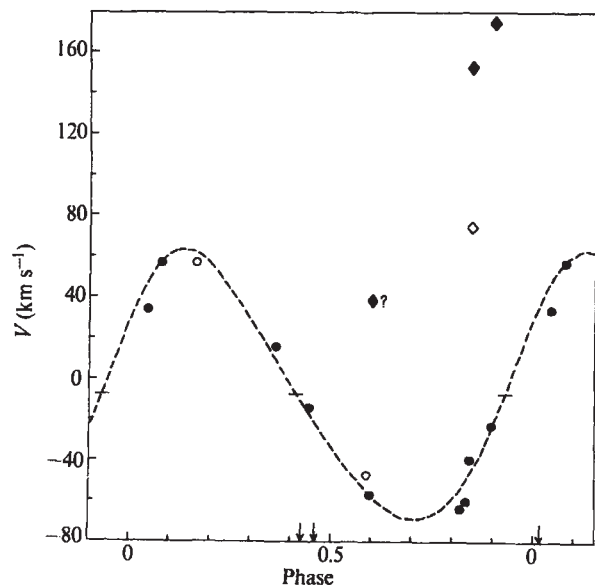


Fig. 1 The radial velocity of HDE 226868 plotted against phase for a 5.607 day period. The circles denote the B star velocities. Open circles indicate uncertain measures. The solid diamonds indicate H β emission line velocities, and the open diamond indicates the secondary component of the K-line measured on one plate. Horizontal tick marks on the velocity curve indicate the centre of mass velocity and therefore the expected eclipse positions. The arrows indicate phases at which photometry was obtained by DuPuy.

of 5.607 days. The orbital elements in Table 2 were derived from this velocity curve. The velocity curve has several notable features. In particular the absorption and emission lines near phase of 0.9 indicate that there is a gas stream moving from the B star toward the unseen companion. The emission line profile is flat-topped suggesting that the feature is a blend of sharper lines having a velocity range of +75 to +350 km s⁻¹ relative to the B star.

The large velocity variation indicates that the ratio of the masses, M_1/M_2 , of the two stars in the system cannot be very large. The mass function

$$f(M) = \frac{M_2^3 \sin^3 i}{(M_1 + M_2)^2} = 0.16$$

requires that $M_2 > 3M_\odot$ if $M_1 \geq 12 M_\odot$. I believe that the latter number is a reasonable lower limit on the mass of the B star. A star could come to have a BOIb spectrum in three ways. The first is through normal stellar evolution, in which case the expected mass would be 12 to 15 $M_\odot$. The second and third are through mass exchange in a binary system³. In the first of these cases a star more massive than 12 to 15 $M_\odot$ could start to exchange mass before the onset of helium burning. Such a star would evolve downwards in the H-R diagram to the right of the main-sequence and could at some point have a BOIb spectrum. It would still have at least 12 to 15 $M_\odot$ at that point, however. A 12 to 15 $M_\odot$ star evolving by mass exchange as above might also have a BOIb spectrum after it had lost 75–80% of its mass. Such a star should show pronounced spectral anomalies, which are not observed in HDE 226868. I believe, therefore, that 12 $M_\odot$ and 3 $M_\odot$ are

Table 2 Preliminary Orbital Elements for HDE 226868

$P = 5.607$ days	$T = 2441214.241$
$V_0 = -7.5$ km s ⁻¹	$T^* = 2441216.554$
$K = 66$ km s ⁻¹	$T^\dagger = 2441219.472$
$e = 0.14$	$a \sin i = 5 \times 10^6$ km = 7.2 $R_\odot$
$\omega = 299^\circ$	$f(M) = 0.16$

* Unseen companion in front of B star.

† Unseen companion behind B star.

reasonable lower limits on the primary and secondary masses, respectively.

The crucial point in identifying HDE 226868 with Cyg X-1 is the comparison of the X-ray period with the velocity period. The radial velocity observations will permit any period within the range 5.595 to 5.635 days. Unfortunately the X-ray data are not so restrictive. Any determination of the X-ray periodicity depends heavily on the minima. At present there are only six well-determined minima in the seven year span of observations. The shortest separation between minima is approximately 6 days so that periods shorter than this cannot be conclusively rejected. Furthermore, the discrete points give no information about the length of the minima, and this permits some leeway in fitting a period to the observations.

Because of these difficulties I have chosen to look for a period within the range allowed by the radial velocities that would fit the X-ray data, rather than to try to determine an independent period for the X-ray data. I have adopted Dolan's interpretation^{2,4} of the X-ray data except for two observations which I explain below. I have also added three data points reported by Matteson⁵.

I have chosen to place in the uncertain category a point that Dolan called a maximum² and another that he called a minimum⁴. The first of these was a point obtained by Brini *et al.*⁶ which was the average of observations on two separate days. The second observation would fall very near a minimum for the period I adopt, and the error bars on the mean of the two observations are large. Therefore I suspect that a maximum and a minimum may have been averaged and the point should be regarded as uncertain. The second observation that I regard as uncertain is one by Agrawal *et al.*⁷ that Dolan interpreted as showing the onset of an eclipse near the end of a 3 h observational run. But Cyg X-3 was also in the field of the detector at the time of the decline. The interpretation of the decline depends on the relative intensity of Cyg X-1 and Cyg X-3. Because no independent information regarding the intensity of the variable source Cyg X-3 is available, this observation should be regarded as uncertain.

I find that a period of 5.607 days fits the X-ray and radial velocity data very well. The X-ray data are plotted against phase for this period in Fig. 2. All but one of the minima fall near primary eclipse (unseen companion in front of B star) and the other minimum is near secondary eclipse (unseen companion behind B star). From the relationship of the X-ray and velocity data it seems that primary eclipse occurs when the unseen companion occults the X-ray region. If the minimum at phase 0.03 is a real eclipse, then its position combined with that of the primary eclipse indicates that the X-ray source is near the unseen companion but trails behind it in the orbit and lies somewhat inside the orbit. More information about the reality and length of secondary eclipse would allow some limits to be placed on the radius of the secondary. Unless the inclination of the orbit is small, the unseen companion cannot be very large because the photometry near primary eclipse (arrows in Fig. 1) indicates that there is no optical eclipse.

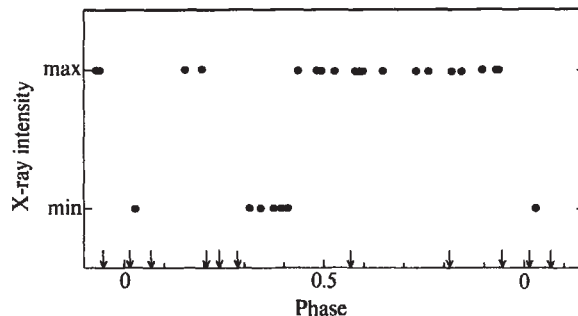


Fig. 2 The X-ray observations plotted against phase for a 5.607 day period. Observations were classified as maximum, minimum, or uncertain. The phases of the uncertain observations are indicated by arrows.

The motion of the gas stream and the apparent position of the X-ray source strongly suggest that the X-rays are being produced through the interaction of the gas stream and the unseen companion. The high energies of the X-rays imply that large accelerations are involved—accelerations such as might be produced by the intense gravitational field of a collapsed object. The lower limits placed on the secondary mass are too high for a white dwarf and probably rule out a neutron star. The lack of a supernova remnant also argues against the secondary being a neutron star. This raises the distinct possibility that the secondary is a black hole. Further observations around secondary eclipse could test this possibility.

The good agreement between the velocity variations of HDE 226868 and the X-ray variations of Cyg X-1, combined with their close positional coincidence, argues strongly for their identity. Furthermore, I have found $\lambda 4686$ of HeII in emission on plates taken by Humphreys, and this and other emission lines may be marginally visible on my plates. This would be very unusual for a BOIb star, but $\lambda 4686$ has been seen in emission in the spectra of Sco X-1 (ref. 8) and Cyg X-2 (ref. 9). Thus the identity of Cyg X-1 and HDE 226868 appears almost certain.

Several problems remain, however. I have already discussed the problems involved in deriving a period for the X-ray variations. The observations by Matteson⁵ suggest that an additional complication may be present. He finds that Cyg X-1 varies by a factor of seven while the observations summarized by Dolan^{2,4} indicate variations of no more than a factor of two. The difference here may be due to some of the experimental problems discussed by Dolan². The difference might also be caused by variations in the synchrotron component of the source. If this were the case, it would be quite easy to derive a spurious period for the X-ray variations from the limited data that are currently available. The true nature of the X-ray variation can only be adequately determined by continuous monitoring of Cyg X-1 over a large energy range. Ideally this should be coordinated with optical and radio observations.

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X Persei an X-ray Source?

We suggest that the peculiar star X Per is connected with the Uhuru X-ray source 2ASE 0352+30.

The sixth magnitude irregular variable X Per (BS 1209=HD 24534), situated in the Per OB2 (II Per) association at $\alpha = 3$ h 52.3 min, $\delta = 30^\circ 54'$ (1950), is of spectral type Opev and shows a large ultraviolet excess^{1,2}. Blaauw³ has suggested that it is the remnant of a supernova explosion that gave rise to the runaway star ξ Per. In a subsequent discussion on the relationship between groupings of early-type stars and X-ray

sources, Braes and Hovenier⁴ predicted that X-ray emission might be observable from the region of Per OB2. Such an X-ray source, 2ASE 0352+30, has now been detected (R. Giacconi *et al.*, unpublished catalogue of Uhuru X-ray sources). The quoted Uhuru position of this source, which has a 2σ uncertainty of $5' \times 15'$, agrees to within $2'$ with that of X Per. These arguments lead us to believe that the X-ray source is associated with this star.

Because some other X-ray sources are known to be radio emitters, we have searched for 1,415 MHz radiation from the X Per region with the Westerbork synthesis radio telescope. Twelve-hour observations made on both May 24 and 27, 1971, failed to detect any source brighter than 0.005 flux units within the 2ASE 0352+30 error box. Because of the extreme radio variability of X-ray sources, however, these measurements are not conclusive. Continued monitoring of X Per at both optical and radio wavelengths is needed.

We thank the ASE group for providing us with data in advance of their publication. The Westerbork Radio Observatory is operated by the Netherlands Foundation for Radio Astronomy with the financial support of the Netherlands Organization for the Advancement of Pure Research (ZWO).

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Possible Identification of X Persei with an X-ray Source

COMPARISON of the Yale catalogue¹ with a recent catalogue of 116 X-ray sources observed with the Uhuru satellite (private communication from R. Giacconi, H. Gursky, E. Kellogg, S. Murray, E. Schreier and H. Tananbaum) shows that the position of the irregular variable star X Persei (see Table 1) coincides with that of the X-ray source 2ASE 0352+30. The total number of chance coincidences of bright stars to within 0.5 arc min in α and $0^\circ.1$ in δ of the Uhuru X-ray source positions is estimated to be ~ 1 —the exact number of chance coincidences is difficult to evaluate because both the bright stars and the X-ray sources have a non-random distribution on the sky—but the very unusual nature of X Persei lends some support to the speculation that the positional agreement of this star with an X-ray source may not be a chance coincidence. The spectrum of X Persei is classified as O pe, and is peculiar because it is strongly veiled.

Table 1 Comparisons of the Positions of X Persei and 2ASE 0352+30

	α_{1950}	δ_{1950}
Optical	3 h 52 m 15 s	$+30^\circ 54'$
X-ray	3 h 52 m 10 s ± 36 s	$+30^\circ 53'$ $\pm 5'$

Quoted errors are 90% confidence limits.

In 1961 Blaauw² suggested that X Persei and the high-velocity O star ξ Persei originally formed a physical binary system which disintegrated when X Persei became a supernova.

The identification of X Persei with an X-ray source would lend considerable support to Blaauw's suggestion.

I thank Professor Giacconi for permission to quote his results in advance of publication.

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Morphology of Carbon Fibres

It has been suggested recently that the morphology of high modulus carbon fibres involves sheet-like elements arranged concentric to the fibre axis^{1,2}, somewhat in the manner of growth rings in trees. We have confirmed this proposal for PAN-based high modulus carbon fibres, supplied by the Celanese Research Company.

Scanning electron micrographs were obtained on a JEOL JSM-2 microscope operated at 25 kV. Fig. 1 shows a scanning

electron micrograph of a fibre broken in tension, in which sheets of the order of 1000 Å thick can be seen. Such a structure was found to be more easily discerned in fibres such as that shown in Fig. 1, which contained a large internal flaw, but this structure was by no means confined to this type of fibre. The structure was demonstrated by breaking the fibre in torsion. In these conditions a major component of the applied strain was taken up as shear between adjacent planes and resulted in a breakdown of the fibre into the sheet-like elements as shown in Fig. 2. This indicates that the cohesive forces within these sheet-like units are greater than those between them.

These sheets are being investigated by transmission electron microscopy and their influence on the mechanical properties of the fibre is being evaluated.

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BIOLOGICAL SCIENCES

Heavy Lysozymuria after X-Irradiation of the Spleen in Human Chronic Myelocytic Leukaemia

By electrophoretic analysis we have found large quantities of an exceptionally basic protein in the urine of five out of seven consecutive cases of chronic myelocytic leukaemia. In four cases the specific proteinuria occurred when induction of remission was achieved after X-irradiation directed against the enlarged spleen. In one case the protein was found before X-ray therapy, but irradiation evoked an increasing protein response. The proteins have been isolated and characterized as relatively low molecular weight constituents with the enzymatic properties of lysozyme.

Twenty-four hour samples of urine were collected and the lysozyme activity was estimated by a slight modification of the method of Osserman and Lawlor¹. The normal range of activity was from 0–2 µg ml.⁻¹. The urine of patients suffering from chronic lymphocytic and acute lymphoblastic leukaemia, acute myelogenous leukaemia and chronic myelocytic leukaemia in general showed activities within the normal range, in agreement with the results of others^{1,2}. High levels of urinary lysozyme activity were observed in acute monocytic and myelomonocytic leukaemia^{1–3}.

After the first course of X-ray therapy, in doses of 4–5 × 100 r., a rapid drop in the number of peripheral leucocytes was observed, with simultaneous increase of urinary lysozyme activity to values in the range of 260–700 µg ml.⁻¹, and was still increased after 45 days. With the development of prolonged

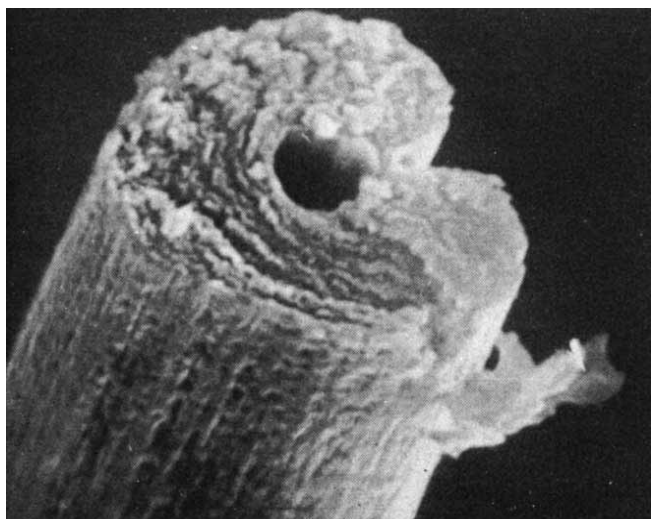


Fig. 1 Carbon fibre broken in tension (×3,750).

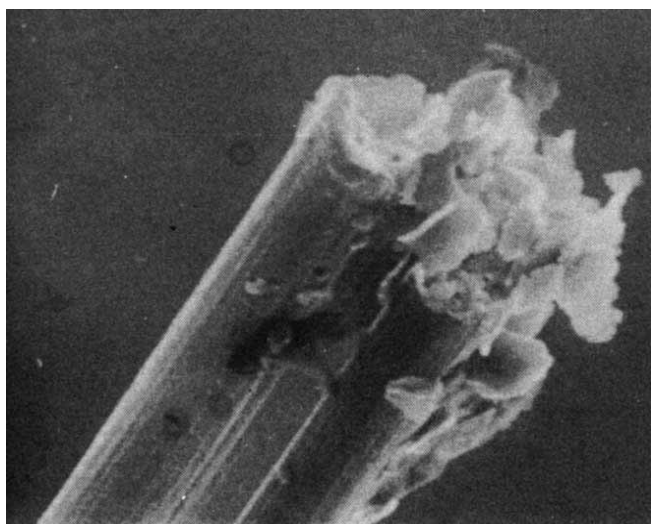


Fig. 2 Carbon fibre broken in torsion (×2,250).

remission, return of the enzyme level to normal was noted. Details of the changes in the blood and urine in a typical case treated by X-irradiation are shown in Fig. 1.

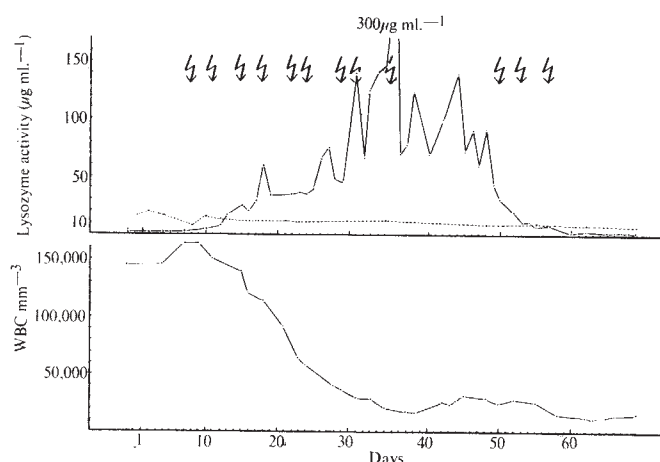


Fig. 1 Changes in the blood and urine of a case of chronic myelocytic leukaemia treated successfully with X-ray irradiation ($\dot{\gamma}$ =100 r.) directed against the enlarged spleen. Lower graph: the rapid decrease of white blood count (WBC); upper graph: the simultaneous increase of urinary lysozyme activity to a maximum value of $300 \mu\text{g ml.}^{-1}$. Fifty days after X-ray therapy, both the differential and the total leucocyte count returned to normal, and the spleen was reduced to less than half its initial bulk. With the development of prolonged remission the urinary enzyme level returned to normal. Serum lysozyme activity (dotted lines) was less significantly affected.

On the basis of the normal renal function studies in most of our patients, it seems that lysozymuria in such patients probably represents a simple threshold phenomenon and is not due to renal tubular dysfunction. All patients with secondary lysozymuria in this study demonstrated reduced or absent leucocyte alkaline phosphatase. There was a decrease in the absolute monocyte counts in the peripheral blood as well as in the bone marrow in the majority of patients. Previous studies have suggested that the serum lysozyme levels are related to granulocyte and possibly monocyte turnover⁴⁻⁶, so the high urinary levels present in our patients are not due to an increased turnover of cells of the monocytic series, but may be due to an increased turnover of the granulocytic series. As other workers showed, the specific granules (lysosomes)^{7,8} of neutrophils, myelocytes and promyelocytes seem to contain the lytic enzyme⁹⁻¹³. In this light, heavy lysozymuria in patients with chronic myelocytic leukaemia obviously mirrors the increased lysosomal breakdown induced by the X-irradiation of the granulocytic cells and their precursors.

Secondary lysozymuria following splenic irradiation is not related to the presence or absence of the Philadelphia chromosome^{14,15} in the granulocytic precursors (F. W. T., G. L., D. M., W. W., and D. Orywall, work in preparation). Of the three patients with secondary lysozymuria one was Philadelphia chromosome positive, two were Philadelphia chromosome negative. Perillie and Finch¹⁶, however, have observed that the rare group of Philadelphia chromosome negative patients with chronic granulocytic leukaemia had primary—not drug or irradiation induced—lysozymuria. This report confirms primary lysozymuria in one case of Philadelphia chromosome negative chronic myelocytic leukaemia and adds the finding of irradiation induced secondary lysozymuria in patients with this disease independent of the absence or presence of the respective chromosomal aberration.

Immunological and peptide fingerprint studies in our laboratory have shown that the lysozyme of one of the cases possessing the Philadelphia chromosome represents the normal enzyme common to all biological fluids. The enzyme was also found to be identical to urinary lysozyme excreted in the rare acute

monocytic leukaemia^{3,17}, where it is produced in amounts up to 2.6 g day^{-1} (ref. 1). It is, however, structurally different from lysozyme of hen's egg white. This experimental evidence permits us to conclude that this readily available "myelocytic" lysozyme may serve as an invaluable source of homogeneous protein for studies designed to elucidate human lysozyme structure and genetic control.

Chronic myelocytic leukaemia occurs relatively frequently; this disease may serve in particular as a source of genetically modified enzyme proteins important in analysing the relationship between amino-acid sequence and enzymatic activity.

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Surface Glycoproteins and Glycolipids of Chicken Embryo Cells transformed by a Temperature-sensitive Mutant of Rous Sarcoma Virus

AMONG the many differences between normal and cancer cells those of the cell surface are most likely to be directly relevant to the altered response of cancer cells to control mechanisms¹⁻¹⁰.

Glycoproteins and glycolipids, important components of the plasma membrane, have been compared in a number of normal and transformed cell systems. The glycolipids of transformed cells have in general a simpler carbohydrate moiety than those of normal cells, probably due to incomplete synthesis¹¹⁻¹⁷. Differences have also been found in the surface glycoproteins of these cells¹⁸⁻²⁴. (Buck *et al.*²² have described a fucose-containing glycoprotein which is much increased in virus-transformed cells.)

We describe here the results of further studies on these glycoproteins using cells transformed by a temperature-sensitive mutant of the Schmidt-Ruppin (subgroup A) strain of Rous sarcoma virus (T5) isolated by Martin²⁵. Chicken embryo cells (CEF) transformed by T5 are like cells transformed by the wild type RSV(SR) at 35°–36° C but lose the transformed morphology at 41° C. Virus production by T5 is the same as the wild type virus at the non-permissive temperature (41° C)²⁵.

We have found that a fucose-containing material in the surface coat is present in increased amount in T5-transformed cells at 35° C compared with untransformed cells but is virtually absent at the non-permissive temperature at which T5 transformed cells closely resemble normal cells. No consistent differences were found in the glycolipids of normal and RSV-transformed cells.

and centrifuged at 1,200*g* for 10 min and the "trypsinase" processed for column chromatography on 'Sephadex G-50' as previously described²².

Fig. 1 shows the results of a study in which ¹⁴C-labelled digests from normal cells are combined with ³H-labelled material from cells transformed by either RSV(SR) or by T5. Cells transformed by RSV(SR) and growing at either 35° C (Fig. 1*a*) or 41° C (Fig. 1*b*) bear material on their surfaces that is larger and elutes earlier than the material in the major peak. This material is obviously heterodisperse. Surface material from cells transformed by T5 and grown at 35° C displays an elution pattern similar to those seen in Fig. 1*a* and *b* but the elution pattern is clearly different when T5-transformed cells are grown at non-permissive temperature. Here the elution patterns of the pronase digests of trypsinates

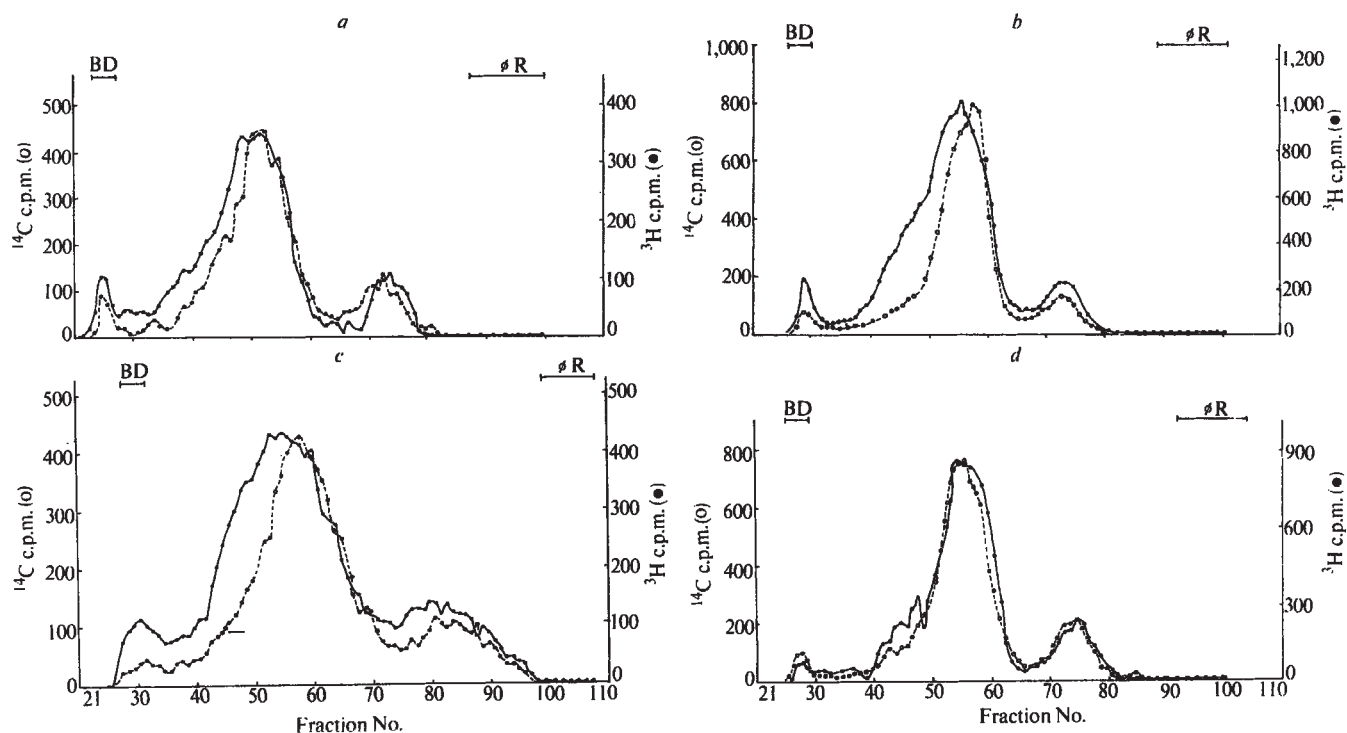


Fig. 1 Co-chromatography on 'Sephadex G-50' of pronase-digested trypsinates from normal cells grown in the presence of ¹⁴C-L-fucose and cells transformed by either RSV(SR) or T5 grown in the presence of ³H-L-fucose. Appropriate trypsinates were combined and digested with pronase for 5 days. Columns of 'Sephadex G-50' (0.8 × 115 cm) were equilibrated and developed with 0.1 M Tris-acetate (pH 9.0), 0.1% SDS, 0.1% mercaptoethanol and 0.01% EDTA. Flow rate was approximately 15 ml/h. Each fraction was 0.7 ml. in volume. BD represents fractions in which the Blue Dextran 2,000 was eluted. φR represents fractions in which phenol red was eluted. *a*, Co-chromatography of pooled, digested fractions from chicken embryo fibroblasts (○) and similar cells transformed by RSV(SR) (●) both grown at 35° C; *b*, the same: both sets of cells grown at 41° C; *c*, co-chromatography of pooled digested fraction from chicken embryo fibroblasts (○) and similar cells transformed by T5 (●): both grown at 35° C; *d*, the same: both grown at 41° C.

Chicken embryo cell cultures were prepared and cultured by methods essentially similar to those described by Vogt²⁶. The embryos were from a stock of C/O Brown Leghorn chickens free from avian lymphomatosis virus. Semi-confluent cultures were infected 18 h after seeding with about 1 focus forming particle per cell and incubated at 37.5° C. Morphological transformation of the cells was apparent after about 5 days and at 10 days most of the cells were rounded and refractile. Cultures in 9 cm polystyrene dishes were subcultured once or twice with a dilution factor of 1 to 5. Radioactive L-fucose was added 1 day after the cells were shifted to 35° C or 41° C. For each cell type, ten to fifteen plates were used containing 6 × 10⁵ cells per dish. Either 50 μCi L-fucose-³H (New England Nuclear, 4.3 Ci/mmol) or 25 μCi L-fucose-1-¹⁴C (Calbiochem, 52 μCi/mmol) was added to each set of plates. The cells were trypsinized 72 h after the addition of isotope and before they became confluent. Individual cell suspensions were pooled

are very close to one another with little evidence of larger surface material which is seen on cells displaying transformed characteristics (T5 at 35° C). The same results have been obtained when the labels were reversed.

A similar double-label experiment has been done with chicken embryo cells infected by RAV1. Extracts of these cells contain a high titre of the avian leucosis group specific antigen by complement fixation. The trypsinates from the surface of normal and RAV1-infected cells are similar showing that the presence of carbohydrate-containing proteins of larger size on the surface of cells is associated with transformation rather than with virus infection (Fig. 2). These results supplement the observations made at 41° C with T5 virus-infected cells in which the virus replicates as efficiently as the RSV(SR) at this temperature.

Normal and transformed cells from the same systems as described for glycoproteins were analysed for their total neutral

lipids, phospholipids and glycolipids by the method of Robbins and Macpherson²⁷. Cells (two plates of each group) were labelled for 3 days at 35° C and 41° C in medium containing 10 μ Ci of 1-¹⁴C palmitate (Radiochemical Centre, Amersham, 57 mCi/mmol). More than 90% of the radioactivity incorporated was extractable into chloroform:methanol (2:1). The total lipid extract was partitioned into organic and aqueous phases; the latter contained 70% of the sialyldihexosyl ceramide (haematoside) and all the higher gangliosides. The radioactively labelled lipids were separated by thin-layer chromatography and the amount of radioactivity incorporated into individual lipids was determined.

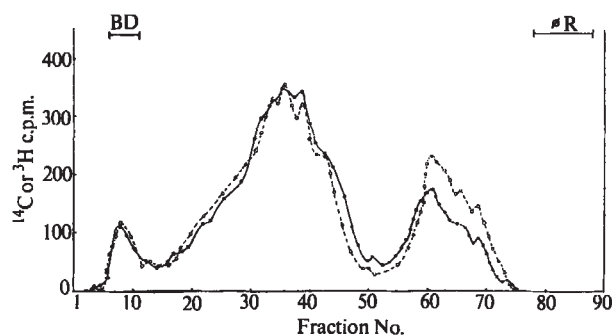


Fig. 2 Co-chromatography on Sephadex G-50' of pronase-digested trypsinates from normal cells grown in the presence of ³H-L-fucose and cells infected by RAV1 virus grown in the presence of ¹⁴C-L-fucose. Procedures as in text and in Fig. 1. Chicken embryo fibroblasts ³H c.p.m. (○); CEF infected with RAV ¹⁴C c.p.m. (●). Both grown at 35° C.

A comparison of the incorporation of 1-¹⁴C palmitate into the lipids of normal and transformed CEF at 35° C and 41° C is shown in Table 1. Within a temperature group, distribution of isotope between the neutral lipid and phospholipid of the three cell lines is fairly constant. The neutral lipid pool varies, however, at the two temperatures presumably because of more rapid utilization of accumulated palmitate at 41° C. No marked difference in incorporation of isotope into glycolipids of CEF and RSV(SR) was found either at 35° C or 41° C.

Table 1 Incorporation of 1-¹⁴C Palmitate into the Lipids of Normal and Transformed Chicken Cells

	CEF	35° C CEF/ RSV	CEF/ T5	CEF	41° C CEF/ RSV	CEF/ T5
Neutral lipid	664*	752	757	325	480	448
Phospholipid	314	228	222	623	470	500
Neutral glycolipid	4.08	4.60	5.50	3.74	4.27	3.41
Haematoside	9.35	5.70	5.25	28.50	22.00	22.70
Higher ganglioside	1.27	0.55	0.81	2.70	2.05	2.12
Ceramide	7.25	8.25	9.40	17.00	21.70	24.00

* C.p.m. $\times 10^{-4}$.

Incorporation of isotope into lipids of the three cell lines has been normalized to a total of 1,000 c.p.m. to enable direct comparisons of distribution to be made. Lipids were separated into total neutral lipid, individual phospholipids and glycolipids by the two-dimensional thin-layer chromatography method of Gray²⁸. Ceramides were separated by two-dimensional thin-layer chromatography on pre-coated silica gel plates (Merck) using chloroform:methanol (95:5 v/v) and ether:chloroform:acetic acid:hexane (55:5:0.15:40 v/v) as solvents. Gangliosides were separated on identical thin-layer chromatography plates using chloroform:methanol:water (60:35:8 v/v) as the solvent. An antioxidant, 26-di-tert-butyl-p-cresol was added to all solvents.

Slight variations seen in some experiments have not been substantiated in others. The T5 transformed cells showed little or no difference from the normal cellular glycolipid pattern at 41° C or at 35° C. The major glycolipids present were the neutral glycolipid ceramide monohexoside and haematoside. Smaller amounts of higher gangliosides were present having retention values on thin-layer chromatography similar to mono and disialogangliosides extracted from sheep brain.

Our data confirm previous results that the surface of virus-transformed cells has relatively large amounts of fucose-containing glycoproteins. It is not known whether this increase is due to enhanced synthesis, decreased degradation, or some other mechanism.

The presence of this material on the surface of T5-transformed chicken embryo fibroblasts at permissive temperature (35° C) and its absence at non-permissive temperature (41° C) correlates with the clear differences in the social behaviour and physiology of the cells at each of these temperatures. Cells at 35° C are rounded and refractile, and form colonies in soft agar²⁵; at 41° C they are elongated and fibroblastic and grow in regular parallel array. They do not form colonies in soft agar²⁵. As it is likely that the temperature sensitive mutation in T5 affects a single gene it is probable that these temperature sensitive changes in the cells are all dependent on this gene. Consequently if tumour induction is likewise controlled by this gene then particular attention should be given to the fucose-containing surface component as a possible key to the changes occurring in surface membrane activities in the malignant cell.

The minimal changes in the glycolipids on transformation of chicken cells with RSV(SR) and T5 at permissive and non-permissive temperatures differ with the results obtained by Hakomori *et al.*¹⁵, also with RSV transformed chicken cells. They reported a decrease in levels of haematoside and higher gangliosides of transformed CEF with concomitant increases in the precursors ceramide monohexoside and ceramide, when expressed on a cell protein basis. The magnitude of these differences, however, was very variable. The failure to detect alterations in the glycolipid pattern on transformation in our study is probably not due to an increase in the incorporation of isotope into lower concentrations of glycolipid as a long labelling period was used. Moreover, the technique has been successfully used to detect alterations in glycolipid metabolism of other transformed cell lines²⁷. The possibility that a smaller proportion of cells in our system were transformed was discounted because ample time was allowed for the virus to multiply and cause repeated infection of the cells.

It may be possible to determine which changes in the surface membrane components of transformed cells are responsible for their altered behaviour by specifically modifying these components enzymatically or serologically and such experiments are being attempted with the fucose-containing glycopeptide in transformed cells.

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***In vitro* Transformation of Syrian Hamster Embryo Cells by Diverse Chemical Carcinogens**

MOST studies of chemical carcinogenesis *in vitro* have been limited to chemicals such as the polycyclic carcinogenic hydrocarbons and 4-nitroquinoline-1-oxide and some of its derivatives (reviewed in ref. 1). With these compounds, cells in culture are transformed *in vitro*, and neoplastic development follows the injection of cells into appropriate hosts. Nitroso compounds such as N-nitrosomethylurea and N-methyl-N-nitrosourethane cause morphological conversion of established cell lines^{2,3}. In one case in which Syrian hamster BHK 21/13C cells had been transformed with N-methyl-N-nitrosourethane, inoculation of cells into hamsters produced tumours at the site of injection; however, when cells not exposed to the chemical were injected, hamsters developed similar tumours which grew more slowly³. No tumours were seen in rats given subcutaneous injections of suspensions of rat fibroblasts whether or not they had been treated with methyl nitrosourethane. Cell multiplication increased in normal hamster cell cultures treated with dimethylnitrosoamine. Transformation was noted only after later passage of cells, and the carcinogen seemed to be responsible for an increase in cell multiplication and cellular life span. Transformation in the cultures treated with dimethylnitrosoamine seemed to result from secondary changes rather than from a direct effect of the carcinogen⁴. N-Nitrosomethylurea in similar circumstances also failed to produce colonies with cells piled up at random as is characteristic of transformation⁴.

More recently, normal Syrian hamster embryo cells have been used to determine directly the frequency of transformation by chemical carcinogens^{5,6}. This phenomenon is inductive rather than selective, as the increase in frequency of transformation on a per cell basis is proportional to concentration (0.1–10 µg/ml. medium)^{7,8}. Furthermore, cells derived from colonies transformed by carcinogenic polycyclic hydrocarbons give rise to cell lines producing tumours when inoculated into hamsters^{1,9}. Our experiments with Syrian hamster embryo cells were undertaken to study whether additional classes of chemical carcinogens could produce transformation and to determine whether transformation could be demonstrated with chemical carcinogens requiring metabolic activation.

The transformation assay has been described previously^{6,8}. Cells from whole embryos of golden hamsters were minced and cultured in Dulbecco's modification of Eagle's medium supplemented with 10% foetal bovine serum. Cells from secondary cultures were cloned at 5×10^2 cells/plate on X-irradiated rat embryo feeder cells in complete medium. Chemicals were dissolved in acetone and added to warm, complete medium to make a stock solution. One day after the cells had been seeded for assay, the concentrates of chemicals, prepared by further dilution of the stock solution with complete medium, were added in amounts to give the final concentration shown in Table 1. Medium was not changed while the dishes were kept in a humidified 10% CO₂ air environment in an incubator at 37° C. Nine days after seeding, the colonies were fixed in methanol, stained with Giemsa, and examined for transformation.

Table 1 Transformation by Chemicals of Various Classes

Compound	Dose †	Treated colonies	Cloning efficiency (%) T/C§	Trans-formants	
				Total	%
Aflatoxin B ₁	0.5	421	5.2/11.4	22	7.0
11-Methylcyclopenta(a)-phenanthrene	5	552	4.6/7.1	32	5.8
N-2-Fluorenylacetamide (FAA)	5	571	4.8/9.2	3	0.5
N-Hydroxy FAA	5	387	3.2/9.2	8	2.1
N-acetoxy FAA	5	532	4.4/9.2	82	15.4
Urethan	50	767	6.4/7.1	0	0
N-hydroxyurethan	12.5	348	2.9/7.1	0	0
Diethylnitrosoamine (DEN)	100	535	8.9/9.0	0	0
N-Methyl-N-nitro-N-nitrosoguanidine (MNNG)	0.5	289	4.8/11.4	21	6.9
Methylazoxymethanol (MAM)*	2.5	660	11/11.4	55	8.4
DEN transplacental	0.08 ‡	674	11/9.0	96	14.3
Urethan transplacental	1 ‡	1,134	18/9.0	204	17.9

* MAM was provided by Dr Marie Spath, National Institute of Arthritis and Metabolic Diseases.

† µg/ml. medium.

‡ mg/g intraperitoneally on day 12 of gestation.

§ T/C, treated colonies/control colonies; no transformation observed in controls.

All plates were seeded from a secondary culture with 5×10^2 cells on a 24 h old rat feeder layer (6×10^4 cells). Chemicals were added 24 h subsequent to seeding hamster cells. Plates were fixed and stained 9 days after seeding. Colonies were scored blind by two observers.

When compounds from seven different classes of chemical carcinogens (Table 1) were tested, transformation, as characterized by colonies with random crisscross pattern of spindle cells not seen in control colonies, was observed in five of seven different classes. Aflatoxin B₁, the most toxic component of the aflatoxin group produced by the mould *Aspergillus flavus*, is a lactone and its chemical structure has been determined.

It is carcinogenic to rat liver, produces congenital malformations in Syrian hamsters, is embryotoxic to rats and mice, and acutely toxic to guinea-pigs^{10,11}. In this experiment, 0.5 µg/ml. was sufficient to produce 7% transformation. A dose-response relationship was noted (Fig. 1). The cyclopenta(a)phenanthrene has strong carcinogenic properties when injected into mice¹². Although closely related sterically to natural oestrogens, this aromatic compound is relatively simple; steroid biochemistry indicates possible pathways by which such compounds could arise *in vivo*, but no such compound thus far has been identified from natural sources. The percentage transformation is similar to that obtained with a number of carcinogenic polycyclic hydrocarbons. The transformation with the aromatic amines tested represents an interesting situation. The parent compound, N-2-fluorenyl-acetamide (FAA), may or may not be carcinogenic, depending on the species of animal tested¹³. FAA is converted by enzymes within cells by hydroxylation to N-hydroxy derivatives. The N-hydroxy FAA may be more active than the parent compound and induce cancer in species not affected by FAA. The N-hydroxy derivative converts to an ester and thus forms the N-acetoxy FAA, which is considered closer to the proximate carcinogenic agent. Results with 5 µg/ml. medium indicated that all compounds reduced cloning efficiency by approximately 50% and thus were toxic; however, the transforming ability is slight with the parent compound and the N-hydroxy derivative, and is greatest with the N-acetoxy derivative. The percentage transformation places the N-acetoxy FAA among the most active carcinogenic agents tested.

Freshly prepared urethan and N-hydroxyurethan failed to transform. N-Hydroxyurethan was toxic when used in a concentration one-quarter that of urethan, which was only slightly toxic and even a ten-fold increase added to mass cultures failed to alter growth rate. Although many attempts have been made to explain urethan carcinogenesis, its mode of action remains unresolved. It has been suggested that urethan's carcinogenic properties result from its conversion into N-hydroxyurethan; however, the most recent study indicates that the chemical specificity of urethan is due to the carbethoxy moiety of the urethan molecule¹⁴.

The findings that diethylnitrosoamine (DEN) is neither toxic nor productive of transformation are consistent with reports on dimethylnitrosoamine in a similar system for bioassay⁴. The *in vivo* activity of nitrosoamine compounds hinges on the conversion by enzymes to an active agent. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is a methylating agent which is a potent mutagen and carcinogen and affects survival of mammalian cells in culture¹⁵. MNNG decomposes slowly at pH 7 with a half-life of more than 8 h in these conditions¹⁶, and, like other nitrosoamides, is an alkylating agent. It differs from DEN in that MNNG is an amide, and its activity obviously does not depend on α hydroxylating enzymes. In its toxicity, it ranks close to 7,12-dimethylbenz(a)anthracene, since a final concentration of 0.5 µg/ml. medium must be used to obtain a significant transformation frequency. A dose-response relationship was noted (Fig. 1) and, because of increased toxicity of MNNG with increased amount of carcinogen, no colonies were observed with 1 µg/ml. medium. Methylazoxymethanol (MAM) is the aglycone of the naturally occurring glucoside cycasin as well as its active component¹⁷. Both compounds are extractable from cycad seeds and have been reported to be hepatotoxic, carcinogenic, teratogenic, and neurotoxic. The metabolites of MAM have not been identified, but azoxymethane may be an important⁵ metabolite for its carcinogenicity. In any event, MAM represents another class of carcinogen which produces transformation *in vitro*. MAM is unique among carcinogens because it can transform without being toxic.

Because of the negative results with urethan and diethylnitrosoamine and because a single treatment with these compounds to appropriate animals results in a high incidence of tumours, we investigated the possibility of *in vivo* activation.

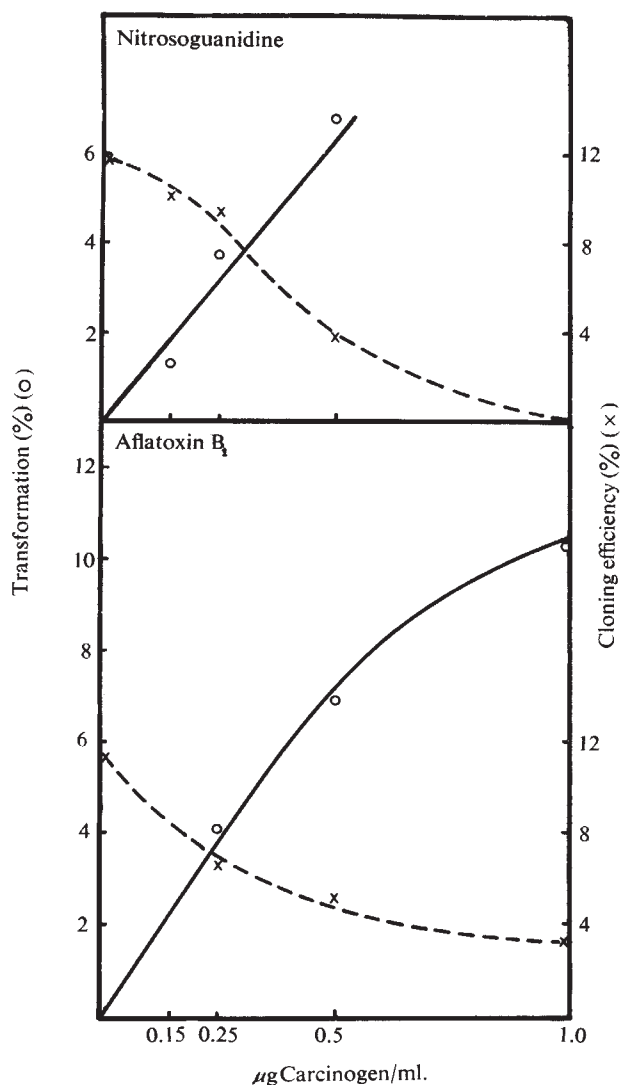


Fig. 1 Relationship of chemical concentration (N-methyl-N'-nitro-N-nitrosoguanidine or aflatoxin B₁) to cloning efficiency and % transformation (transformed colonies/total colonies). Experimental details are as described in Table 1. Ten plates seeded for each point shown. Nitrosoguanidine could not be assessed for transforming activity at 1 µg/ml. because cytotoxicity destroyed the cells.

The bioassay system was modified by intraperitoneal injection of urethan or DEN into pregnant hamsters on day 12 of gestation, and the embryos were removed on day 14, a period that allowed for completion of metabolic interaction. When the cells were prepared as usual and no chemical was added, transformation was extremely significant. With urethan, two transformed colonies were isolated and cell lines developed which, when injected into animals, produced fibrosarcomatous tumours. It was thus possible to demonstrate transformation by chemicals requiring activation.

Transformation by different classes of carcinogens is significant because cell lines from transformed colonies produced tumours when injected into animals. Furthermore, we have reported that cultures of primary tumours induced by carcinogens *in vivo* in hamsters have colonies with overlapping cellular elements similar to those found after treatment of normal cells with carcinogenic chemicals¹⁸.

When a number of carcinogenic polycyclic hydrocarbons were tested for transformation, they could be classified as highly oncogenic, weakly oncogenic, or non-oncogenic by their known tumorigenicity in intact animals⁶. Because of intracellular transport problems and other unknown complications, the correlation of oncogenicity *in vivo* with a chemical trans-

formation property *in vitro* may not always be possible. Furthermore, as with some compounds tested when injected or fed to animals, organ specificity of carcinogens must be considered. Some carcinogens are multipotential, while others are extremely specific in terms of the target organ affected. The latter results may reflect method of application or may have involved tumours produced first in the largest number of animals and responsible for the death of the animals before the other tumours became recognizable. In some instances, all target sites may not have been examined in detail. The nature of the target cell *in vitro*, because of its genetic history, may have influenced the type of tumour produced by chemical transformation.

We propose that *in vitro* models can be used for the study of chemical carcinogenesis. Existing models can be adapted and new models devised for the study of the metabolism of chemical carcinogens and for the screening of human environmental agents.

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Production of Interferon by Ultraviolet Radiation Inactivated Newcastle Disease Virus

MYXOVIRUSES, irradiated with ultraviolet light, are good inducers of interferon formation in both chick and L cells¹⁻⁴. Unirradiated virus does not induce interferon formation in chick cells¹⁻³, but does so in mouse L cells⁴, and, in both systems, inducing capacity is lost on prolonged irradiation. Three questions therefore may be asked: (1) why do infective Newcastle disease virus (NDV) and influenza virus fail to provoke interferon formation in chick cells; (2) by what mechanism does irradiated, non-infective, virus produce interferon; and (3) why is this capacity lost on prolonged irradiation?

Several lines of evidence suggest that the unirradiated myxoviruses are intrinsically capable of inducing interferon formation. A large number of unirradiated NDV strains produce interferon in chick cells if the cells have been pre-treated with interferon⁵, while the H strain will produce low titres of interferon without interferon pretreatment⁵, and unirradiated NDV will produce interferon in L cells^{4,6}. If infective virus is therefore capable of inducing interferon formation in certain conditions, why does not it do so in all? Gandhi and Burke⁷ suggested that unirradiated myxoviruses failed to produce interferon in chick cells because infection led to an inhibition of cellular RNA and protein synthesis, and hence of interferon production. They found that when virus was irradiated it lost its capacity to depress host cell RNA and protein synthesis, and that interferon was not formed until virus was sufficiently irradiated to have no effect on these functions. L cells, however, treated with NDV produced interferon in spite of the fact that the inhibition of cellular RNA and protein synthesis was more marked than in chick cells⁶ (S. S. Gandhi and D. C. B., unpublished observations), suggesting that this inhibitory effect of virus infection on cellular RNA and protein synthesis was not responsible for the control of interferon production. This was confirmed by the results obtained with avirulent strains of NDV—which, although they did not inhibit cellular RNA and protein synthesis, did not produce interferon⁵. Thus, interferon is only produced by unirradiated virus either when it multiplies poorly (as in L cells) or if the cells are pretreated with interferon. As interferon acts by inhibiting viral protein synthesis⁸, we suggest that the failure of unirradiated virus to produce interferon is due to the production of a viral protein which inhibits interferon formation, and recent work in this laboratory (E. T. Sheaff and D. C. B., unpublished observations), in which mixing of infective virus with non-infective virus caused an inhibition of interferon formation, supports this suggestion.

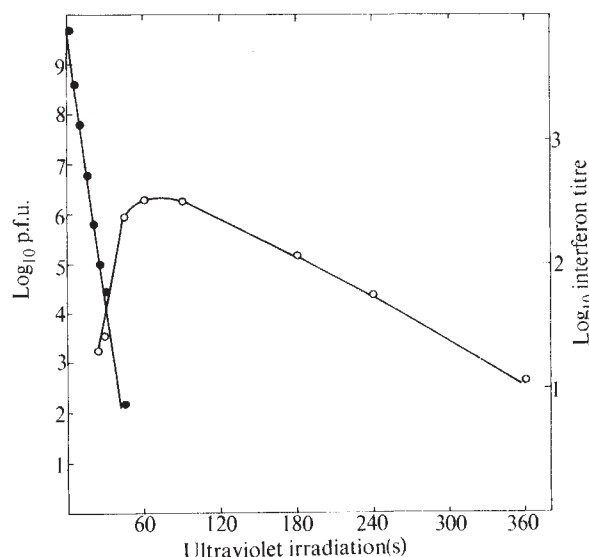


Fig. 1 The effect of ultraviolet irradiation on the infectivity (●), and interferon-inducing capacity (○), of purified NDV (Texas strain). The preparation of media, cells and viruses has been previously described²⁵. Purified virus²⁶ was irradiated as described by Long and Burke²⁷ using a dose of 61 erg/mm²/s.

The capacity of irradiated, non-infective viruses to produce interferon is restricted to the influenza, para-influenza and reoviruses^{1-3,9}. All three groups contain RNA-directed RNA polymerases in the virion¹⁰⁻¹², and we now report an investigation of the role of the virion RNA and virion polymerase in interferon production.

We first investigated the effect of ultraviolet irradiation on the viral nucleic acid to see if the loss of interferon-inducing

capacity after long periods of irradiation was due to breakdown of the viral nucleic acid. When NDV (the virulent Texas strain) was irradiated with ultraviolet light, infectivity was lost with first-order kinetics (Fig. 1). Interferon production rose to a maximum after 60 s irradiation and then declined⁷. Extraction of the viral nucleic acid, followed by fractionation by polyacrylamide gel electrophoresis, showed that the nucleic acid of virus which had been irradiated for longer than 60 s was progressively broken down to smaller pieces (Fig. 2). When the viral nucleic acid, at the same concentration, was similarly irradiated, however, there was no breakdown after 6 min irradiation, but breakdown did occur in the purified viral ribonucleoprotein and 'Triton N101'-treated particle (Fig. 3). Both the viral nucleoprotein and the 'Triton' treated particle showed some polymerase activity before ultraviolet irradiation. As the amount of ultraviolet light is insufficient to cause direct breakage of covalent bonds of the inter-nucleotide linkages¹³, the involvement of an enzymatic process was indicated. It remains to be determined whether the breakdown results from contaminating nucleases¹⁴ or whether the RNA polymerase is involved.

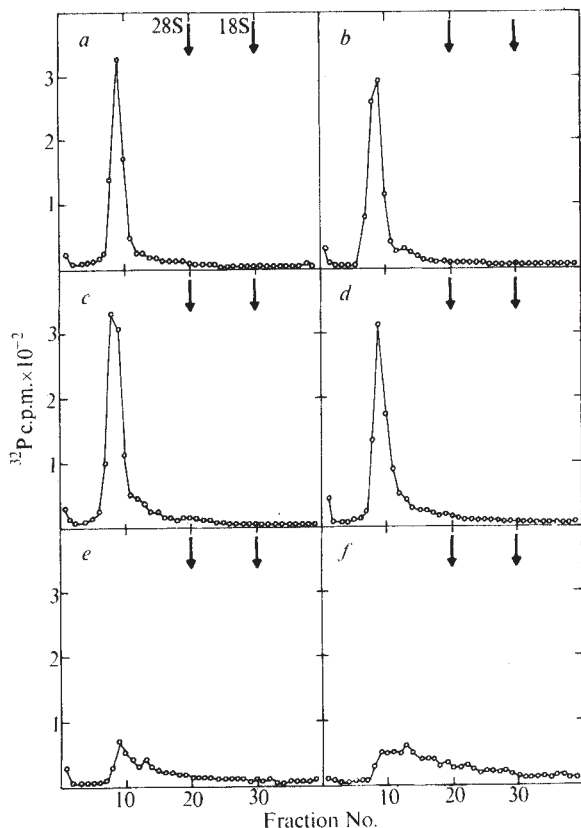


Fig. 2 The effect of ultraviolet irradiation on the molecular weight of RNA extracted from the Texas strain of NDV. Virus nucleic acid was extracted from purified irradiated virus and fractionated by polyacrylamide gel electrophoresis as described by Lomniczi, Meager and Burke²⁶. The arrows show the position of the ribosomal markers. Two other strains (F and H) gave similar results. *a*, Unirradiated control; *b*, 15 s irradiation; *c*, 30 s irradiation; *d*, 60 s irradiation; *e*, 180 s irradiation; *f*, 360 s irradiation.

The effect of ultraviolet irradiation on the activity of the enzyme was then investigated. Ultraviolet irradiation led to an initial rapid loss in polymerase activity followed by a slower decline (Fig. 4). Comparison of Figs. 1 and 4 showed that the slow decline in polymerase activity paralleled the loss in interferon-inducing capacity, suggesting that the two were possibly connected.

Why is polymerase activity lost less rapidly than infectivity on ultraviolet irradiation of the virus? The initial effect of ultraviolet irradiation on RNA viruses is to cause formation of uracil hydrate and possibly uracil-dimers¹⁵⁻¹⁷. In the case of viruses like NDV, which carry their own polymerase, these lesions are likely to block the progress of the polymerase¹⁸, thereby preventing complete transcription of the RNA. As it is the newly synthesized strand that is translated in the polysomes¹⁹, viral protein synthesis is inhibited and no infective virus is produced. Thus, there may be some viral RNA synthesis without subsequent translation.

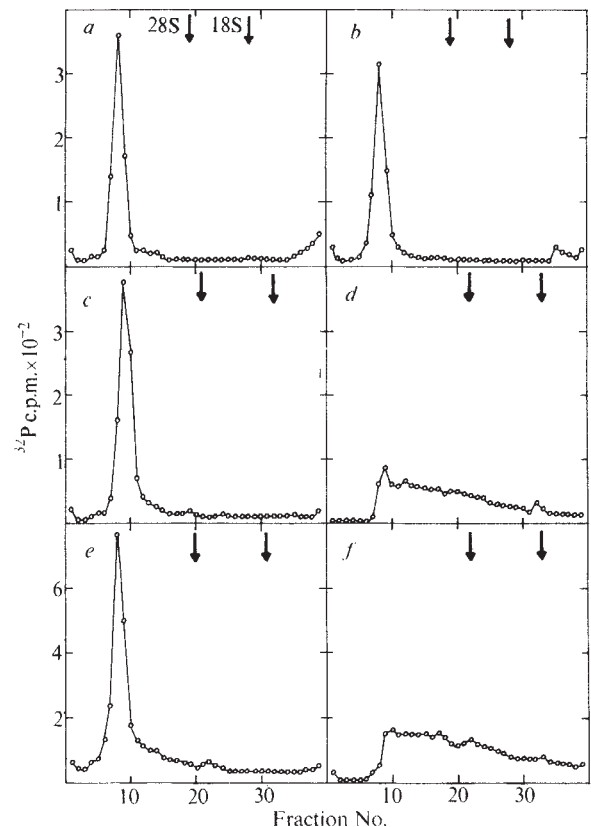


Fig. 3 The effect of ultraviolet light on the molecular weight of NDV RNA, and on the RNA extracted from the viral nucleoprotein and the 'Triton N101'-treated particle. The nucleic acid was fractionated by polyacrylamide gel electrophoresis before (*a*, *c* and *e*) and after 360 s irradiation (*b*, *d* and *f*). Viral nucleic acid was extracted from the virion before irradiation (*a* and *b*), or from the viral nucleoprotein obtained from purified virus according to the method of Mountcastle *et al.*²⁸ (*c* and *d*), or from the particle obtained by treatment of the virion with 0.08% v/v 'Triton N101' at 37° C for 20 min (*e* and *f*). The latter was separated from detergent by pelleting through a 3 ml. cushion of 20% w/v sucrose in an MSE 10×10 ml. angle rotor at 35,000 r.p.m. for 45 min at 4° C. The arrows show the position of the ribosomal markers.

Why does interferon producing capacity seem to be connected with an undegraded viral nucleic acid molecule and retention of polymerase activity? Three possible explanations may be suggested. First, interferon production requires the formation of double stranded RNA. It is known that in both NDV and in the similar vesicular stomatitis virus infection, the newly synthesized RNA molecule is associated with the template RNA of the virus, and a partially double stranded structure has been isolated from the *in vitro* RNA polymerase system^{11,20}. This double stranded structure is associated with the virus particle, and on longer periods of incubation newly synthesized single stranded RNA is released from the particle^{20,21}. This double stranded structure may well arise by self-hybridization of plus and minus strands during phenol

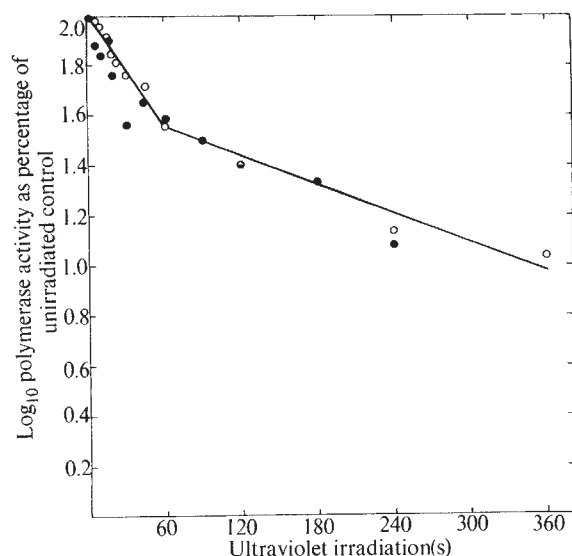


Fig. 4 The effect of ultraviolet irradiation on the NDV RNA directed RNA polymerase. The enzyme was assayed as described by Huang, Baltimore and Bratt¹¹. Two experiments are shown, the specific activity of the unirradiated virus being 1.2×10^4 c.p.m./mg protein (○), and 0.79×10^4 c.p.m./mg protein (●).

extraction²², but even if it is formed *in vivo*, it may be inside the virus particle and we consider that, for these reasons, it is unlikely to be an interferon inducer. Second, interferon formation requires an undegraded viral RNA molecule, and when this is broken down both interferon-inducing capacity and polymerase activity are lost. A third explanation is that interferon production is a consequence of limited viral RNA synthesis, possibly because the association of a host factor with the viral polymerase (leading to enhancement of polymerase activity²³) triggers the sequence of events leading to derepression of the interferon gene. Further work is necessary to distinguish between these possibilities.

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Cross-linking of Complementary Strands of DNA in Mammalian Cells by Antitumour Platinum Compounds

Cis platinum (II) diammino-dichloride, *cis* Pt(II)(NH₃)₂Cl₂ (A), is very effective against sarcoma 180 and leukaemia L 1210 in mice^{1,2} as well as Dunning ascites leukaemia and Walker 256 carcinosarcoma tumours in rats³. This and related compounds also reversibly inhibit cell division and induce cell elongation in *E. coli* B^{4,5}. At a concentration below 5 μM, *cis* Pt(II)(NH₃)₂Cl₂ selectively inhibits DNA synthesis in human amnion AV₃ cells⁶, and there is a correlation between the relative effectiveness of a series of compounds and their capacity to inhibit DNA synthesis⁶. Support for the suggestion that in this cell line the effective platinum compounds acted by binding directly to DNA was obtained by Howle and Gale⁷. They found that DNA synthesis was selectively and persistently inhibited in Ehrlich ascites tumour cells removed from rats up to 4 days after treatment with a single injection of 10 mg/kg of A.

There seems to be a parallel between the properties of this class of compound and of classical antitumour difunctional alkylating agents (ref. 8 and personal communication from T. A. Connors, A. Haddow and W. C. J. Ross) which are thought to react with DNA to form interstrand cross-links⁹⁻¹², thus selectively inhibiting DNA synthesis. Moreover, at concentrations permitting high survival of mammalian cells in culture this was the only detectable biochemical lesion¹³⁻¹⁵. We have recently developed a new and unequivocal method for demonstrating interstrand cross-links in the DNA of HeLa cells treated with mustard gas¹⁶. We now report similar evidence that compound A can also cross-link complementary strands of DNA, which substantiates the view that both types of agent owe their cytotoxic properties to cross-linking of complementary DNA strands.

To detect cross-linking, one strand of DNA was given a density label by growing cells in the presence of 5-bromo-2'-deoxyuridine (BUdR). Cross-linking between a "heavy" and a "light" strand of DNA then produced a "hybrid" species consisting of equal amounts of heavy and light DNA. When such DNA was subjected to alkaline caesium chloride gradient centrifugation heavy, light and hybrid DNA was separated. DNA isolated from cells grown for one generation (approximately 24 h) in medium containing BUdR (5 μg/ml.) and subjected to such isopycnic sedimentation had two ultraviolet absorbing peaks of equal size corresponding to single stranded heavy and single stranded light DNA, and when isolated from cells which had been treated with the platinum compounds or mustard gas there was a peak of DNA of intermediate density. Although this method demonstrated the existence of cross-links in cellular DNA treated with platinum compounds or mustard gas, quantitation of the extent of cross-linking was not possible. For this, the method had to be modified¹⁶. Heavy DNA was given a radioactive label such that the hybrid DNA could be accurately assayed as a percentage of the heavy DNA

peak. Concomitant radioactive and density labelling of DNA was achieved by growing cells in a mixture of BUdR and ^3H -TdR which was equivalent to ^3H -BUdR in producing labelled heavy DNA¹⁷. Cells were grown in the presence of BUdR (5 $\mu\text{g}/\text{ml}$.) and ^3H -TdR for 3 h and to ensure that no radioactive thymidine entered any light DNA they were grown for 2 h before and 1 h after this treatment in the presence of BUdR (5 $\mu\text{g}/\text{ml}$.) only. After this treatment the bulk of the

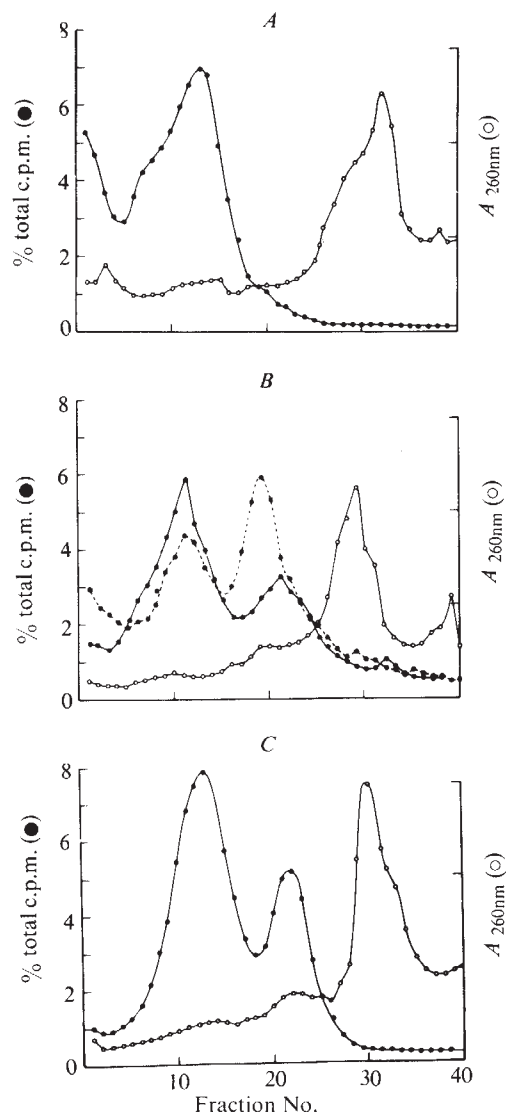


Fig. 1 Formation of cross-linked DNA in HeLa cells treated with *cis* Pt(II)(NH₃)₂Cl₂ and mustard gas. HeLa cells were grown as a stirred suspension culture in Eagle's medium supplemented with 7% foetal calf serum at a concentration of 4×10^5 cells/ml. in the presence of BUdR (5 $\mu\text{g}/\text{ml}$.) for 2 h, then in medium containing BUdR (5 $\mu\text{g}/\text{ml}$.) and ^3H -TdR (1 $\mu\text{Ci}/\text{ml}$.; 26 Ci/mmol.) at a cell density of $8 \times 10^5/\text{ml}$. for 3 h. Cells were collected by sedimentation and resuspended in medium containing BUdR (5 $\mu\text{g}/\text{ml}$.) only for 1 h before treatment with *cis* Pt(II)(NH₃)₂Cl₂ in DMSO (0.5%) or mustard gas in ether (0.02%). Cells were collected by sedimentation 30 min after the addition of mustard gas or 2 h after the addition of the platinum compound and washed with saline. DNA was isolated by the phenol/*p*-aminosalicylic acid method¹⁹ with minimum shaking and subjected to alkaline caesium chloride gradient centrifugation as described¹⁷. Eight drop fractions were diluted with water (0.5 ml.) for measurement of absorbance at 260 nm and then sampled for determination of radioactivity. Counts were plotted as a percentage of total counts on the gradient. **A**, Control, DMSO only. ●, c.p.m.; ○, absorbance 260 nm. **B**, 500 μM and 1,000 μM *cis* Pt(II)(NH₃)₂Cl₂. ●—●, c.p.m., 500 μM ; ●—●, c.p.m., 1,000 μM ; ○—○, absorbance 260 nm, 1,000 μM . **C**, Mustard gas, 2 $\mu\text{g}/\text{ml}$. ●, c.p.m.; ○, absorbance 260 nm.

DNA was light and detected on the gradient by its ultraviolet absorption. Heavy DNA was detected by its radioactive label and, less sensitively, by its ultraviolet absorption (Fig. 1A). After treatment with the platinum compound a new labelled peak of intermediate density appeared on the gradient between labelled heavy and light DNA (Fig. 1B) similar in pattern to that previously observed in cells treated with mustard gas (Fig. 1C). The amount of hybrid DNA was found to be directly dependent on the dose of the platinum compound or mustard gas. The amount of cross-linked DNA determined from the area under the hybrid peak was expressed as a percentage of total heavy DNA in the control and plotted as a function of dose to give the dose-dependent relationship illustrated in Fig. 2. The derivation of this hybrid peak from heavy DNA was confirmed by plotting the loss of heavy DNA as a function of concentration of platinum compound to give a similar relationship (Fig. 2).

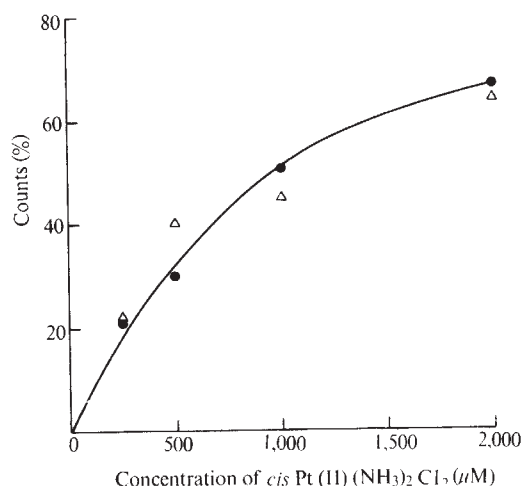


Fig. 2 Relationship between concentration of *cis* Pt(II)(NH₃)₂Cl₂ and extent of cross-linking of heavy DNA. The amount of cross-linked material (counts under the hybrid peak above control) was expressed as a percentage of total counts on the gradients and plotted as a function of dose of platinum compound (●). The percentage loss of material from the heavy peak was similarly plotted against concentration of A (Δ). ●, Percentage of cross-linked DNA; Δ, percentage lost from heavy DNA.

The platinum compounds can react with a variety of nucleophilic centres such as are found in proteins⁸. It is possible therefore that the cross-linking which has been observed is a consequence of a complex reaction involving nucleoprotein and platinum, which prevents separation of the DNA strands. This was precluded by isolating DNA from cells which had been given the same radioactive and density labels as in the previous experiment (Fig. 1) and treating this *in vitro* with various concentrations of A. Cross-linking of heavy to light DNA was similarly observed in these conditions and could not have involved the participation of protein. The colony-forming ability of HeLa cells following 2 h treatments with varying concentrations of A enabled construction of a dose-survival curve. This was a rectilinear curve with an initial shoulder, of slope (D_0) of 3 μM and D_0 of 2 μM . An extrapolation of the initial linear portion of the curve relating cross-linking to concentration of A as shown in Fig. 2 indicates that at a concentration of 3 μM approximately 0.2% of the DNA is cross-linked. Clearly the percentage cross-linking of heavy and light strands in a given situation depends on the molecular weight of the DNA isolated. If this is decreased by shearing, the percentage cross-linking will apparently decrease. The molecular weight of DNA as isolated is approximately 2×10^7 while that in the nucleus is probably not less than 2×10^8 (ref. 18). Hence the actual extent of cross-linking in the cell at a given dose is at

least ten times that shown in Fig. 2. Therefore at a dose of 3 μM A approximately 2% of the DNA is cross-linked, a result of the same order as the corrected value of 10% cross-linking observed at the D_0 dose (0.05 $\mu\text{g ml.}$) of mustard gas¹⁶.

It is currently believed that cross-linking of guanine bases in complementary strands of DNA is responsible for the cytotoxic action of difunctional alkylating agents and the models show that alkylating centres must be 5–7 Å apart for this to occur. For the *cis* platinum compound to cross-link the functional centres must be 2.8 Å apart. There are several potentially reactive centres in DNA including the amino-groups of DNA bases (personal communication from S. Mansy and A. J. Thomson). A model of DNA shows clearly that such amino-groups on adjacent complementary base pairs are conveniently placed for cross-linking. This is particularly apparent for the 6-amino-groups of adenine in an ApT sequence, for the 2-amino-groups of guanine in the narrow groove or the 6-amino-groups of cytosine in the wide groove of DNA of a CpG sequence.

Similar evidence for cross-linking to that presented here was obtained when HeLa cells were treated with another potent cytotoxic platinum compound, *cis* platinum (II) ethylenediamine dichloride, at a concentration of 500 μM for 2 h.

There is evidence that alkylated DNA can be repaired^{14,15} and it is possible that differences in such repair ability could account for the resistance which some cell types exhibit towards alkylating agents. Future studies will therefore attempt to elucidate the sites of attack of platinum compounds in DNA and determine whether any of these are similarly repaired.

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⁵⁵Fe in Human Blood in Norway 1967-1969

⁵⁵Fe has gained increased attention as a fallout isotope during recent years. With low energy 5.9 keV X-rays and a comparatively short half-life of 2.60 yr, this isotope would be relatively harmless to living organisms without being concentrated. An accumulation of iron occurs naturally, however, and human uptake and loss are small compared with total body content. Two main ecological routes for accumulation of ⁵⁵Fe in man have been reported: a sea-plankton-fish-man chain, and a lichen-caribou-Eskimo chain in Alaska¹⁻⁴. High concentrations are also found among Finnish⁵⁻⁶ and Swedish⁷ Lapps.

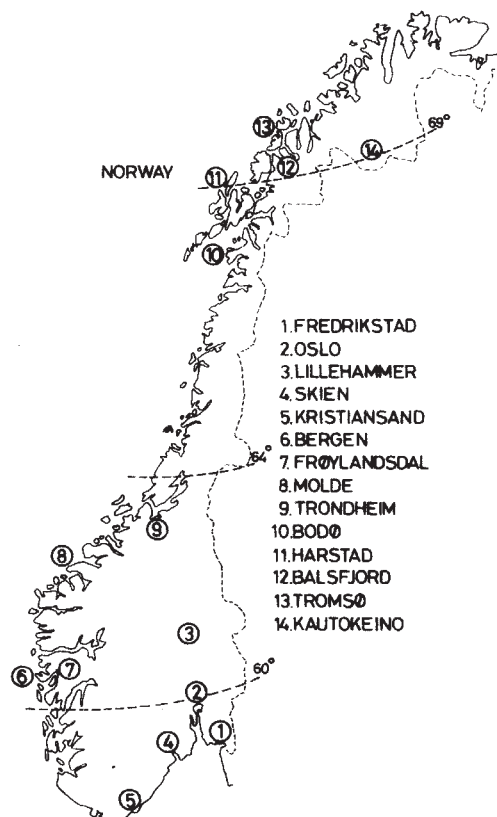


Fig. 1 Map of Norway indicating collecting sites.

Blood samples were collected from Norwegians at fourteen different locations (Fig. 1). Samples from sites 12 and 14 are from Lapps. Sites 10 and 13 are northern coastal cities with good access to ocean fish. Site 7 is characterized by high concentrations of ⁸⁹Sr, ⁹⁰Sr and ¹³⁷Cs in humans and in milk products due to a combination of extensive farming methods and high precipitation amounts. Site 3 is a typical inland agricultural district. The blood samples were mostly collected from regional hospital blood banks; 300-ml. portions were dry-ashed and the ashes dissolved in hydrochloric acid (1 N). Iron was extracted using an anion-exchange column ('Dowex 1X-8') followed by electro-deposition on copper plates. A multichannel analyser was used for counting (Table 1).

The measurements were found to be normally distributed around the population means ($\chi^2 = 1.475$ by goodness-of-fit test). There is no obvious correlation between ⁵⁵Fe in blood and precipitation during the probable period of accumulation, 1961–1966. (In this respect ⁵⁵Fe differs considerably from other fallout isotopes such as ¹³⁷Cs and ⁹⁰Sr^{8,9}.) Delayed fallout is expected to be equally distributed in precipitation, so the results from sites 7 and 14 show that accumulation must have occurred. Lapps consume relatively large quantities of

reindeer meat, and the five males at site 12 are typical examples. The eleven persons at site 14 constitute a more varied group; typical Lapps, as far as consumption of reindeer meat is concerned, have ^{55}Fe concentrations around 20 nCi/l., whereas more urbanized Lapps show values around 10 nCi/l. The Norwegian measurements agree well with Finnish measurements⁶.

Site 13 has significantly lower values than sites 12 and 14 ($P < 0.001$ by t -test), and significantly higher values than sites 10 ($P < 0.01$) and 6 ($P < 0.005$). It is interesting to compare these three coastal cities (sites 13, 10 and 6), for *per capita* consumption of the abundant supplies of ocean fish is assumed to be almost the same. The significant differences cannot be

This is ten times higher than usual values in Southern Norway in 1969.

The range of low energy K X-rays emitted from ^{55}Fe in tissue is about 1 mm^{7,11}. Consequently, there is scant possibility of external exposure, except through skin lesions. The highest internal concentrations of iron are in the blood, mainly inside erythrocytes. Integrated into the haemoglobin molecule, the iron follows the individual erythrocyte during its life span of 120 days. The total dose to the erythrocytes is from K X-rays and Auger electrons. The latter are absorbed almost completely inside the 7- μm erythrocytes, the free path in tissue being about 1 μm ¹¹. Thus the dose to the erythrocytes is slightly higher than to the adjacent plasma, other corpuscles

Table 1 ^{55}Fe in Human Blood in Norway

Year	Site	<i>n</i>	Males Mean $\pm$ s.e. nCi/l.	<i>n</i>	Females Mean $\pm$ s.e. nCi/l.	Population mean nCi/l.	Precipitation 1961–1966 1,000 mm
1967	1 Fredrikstad	9	5.5 $\pm$ 1.1	3	5.1 $\pm$ 1.3	5.3	
	4 Skien	7	4.0 $\pm$ 0.7	3	2.2 $\pm$ 0.1	3.1	
	5 Kristiansand	4	5.0 $\pm$ 1.1	1	6.9	6.0	
	6 Bergen	8	5.9 $\pm$ 0.7	0		5.9	
	8 Molde	3	3.9 $\pm$ 1.1	1	4.8	4.3	
	9 Trondheim	8	5.3 $\pm$ 0.7	2	2.6	4.0	
	11 Harstad	9	9.7 $\pm$ 1.1	3	8.5 $\pm$ 1.6	9.1	
1969	2 Oslo	30	2.55 $\pm$ 0.20	20	2.27 $\pm$ 0.26	2.41	4.9
	3 Lillehammer	17	3.45 $\pm$ 0.61	10	2.58 $\pm$ 0.26	3.02	4.7
	5 Kristiansand	34	2.48 $\pm$ 0.14	10	2.00 $\pm$ 0.14	2.24	8.4
	6 Bergen	25	3.52 $\pm$ 0.22	19	2.14 $\pm$ 0.22	2.83	11.5
	7 Frøylandsdal	7	3.5 $\pm$ 0.6	4	3.2 $\pm$ 0.8	3.4	18.3
	10 Bodö	21	4.70 $\pm$ 0.38	3	4.4 $\pm$ 0.1	4.55	6.6
	12 Balsfjord	5	19.0 $\pm$ 0.8	0		19.0	
	13 Tromsö	22	6.10 $\pm$ 0.45	12	4.97 $\pm$ 0.51	5.54	6.5
	14 Kautokeino	8	12.8 $\pm$ 2.6	3	11.0 $\pm$ 3.2	3.2	2.2
	Total	217		94			

Mean = the arithmetic mean of n blood samples. s.e. = standard error of mean. Population mean = the estimated mean for a population containing equal numbers of males and females. Precipitation amounts during the period 1961–1966 are listed in the right-hand column to allow direct comparison with ^{55}Fe values.

fully explained from this study, but three possibilities are proposed. First, the northern cities might have a particularly high *per capita* consumption of reindeer meat^{5–7}; second, consumption of fish from the polar seas might be exceptionally high²; and third, some unknown ecological routes could be present. Results from sites 5 and 6 allow direct comparison between blood levels in 1967 and 1969. Reduction to 50% is estimated as taking 2.0–2.5 yr. Because ^{55}Fe concentrations in surface air during 1967–1969 are only a tenth of those in 1964¹⁰, the supply to humans during this period can be disregarded, and the biological half-life for ^{55}Fe in human blood must consequently be comparatively long.

The daily loss of stable iron to be replaced in the diet is about 1 mg for males and 2 mg for females. About 6 and 3 yr respectively are needed to reduce total body iron content to 50% of the normal value of 4.5 g if the intake is terminated.

If total blood volume is 6 l., and stable iron in blood comprises 65% of total body iron, the body burden of ^{55}Fe is

$$B = 9.2 A \text{ (nCi)}$$

where A is the ^{55}Fe concentration in whole blood (nCi/l.). Mean body burdens vary from 21 nCi at site 5 to 175 nCi at site 12. The highest value measured in this investigation, 28.9 nCi/l. in a Lapp, corresponds to a body burden of 265 nCi.

and vessel walls. According to the formula given by Wrenn and Cohen¹¹, the total dose rate to the erythrocytes is

$$D = 1.83 \text{ rad/week/pCi/mg Fe}$$

This gives about 0.08 mrad yr⁻¹ at site 5, and for the Lapps about 1.1 mrad yr⁻¹ (RBE = 1). Persson's⁷ formula gives the annual dose to the erythrocytes as 0.106 mrad/pCi/mg Fe, compared with 0.095 when applying the formula of Wrenn and Cohen¹¹.

Mature erythrocytes might be expected to be almost insensitive to ionizing radiation, because they perform no more cell divisions and have a slow metabolic rate. The effects on spleen and blood forming red bone marrow should therefore be considered more closely. The spleen weighs about 150 g and contains about 100 ml. blood. Assuming a spleen content of 70 mg iron, the dose for 1969 will be about 0.001 mrad yr⁻¹ for Southern Norway and 0.02 mrad yr⁻¹ for the Lapp with the highest measured activity. Red bone marrow weighs about 1,500 g. With an iron content of 550 mg, the dose rate will be about 0.01 mrad yr⁻¹ for Southern Norway and 0.15 mrad yr⁻¹ for Lapps.

The proteins ferritin and haemosiderin have about 20% by weight of stored iron. According to Persson they receive annual doses of 13 and 20 mrad/pCi/mg Fe, respectively⁷. For

Southern Norway and the Lapps, respectively, the annual absorbed dose to ferritin was 60 and 730 mrad, and to haemosiderin 100 and 1,210 mrad. The 1969 doses (erg) for sites 5 and 14 are calculated as: whole body, 2.1 and 25.3 g rad; whole blood, 1.4 and 16.6 g rad; erythrocytes, 1.3 and 15.3 g rad; red bone marrow, 0.05 and 0.6 g rad; ferritin and haemosiderin, 0.014 and 0.17 g rad, respectively. In 1969 males have higher ^{55}Fe concentrations than females ($P < 0.002$ by t -test). This is in agreement with Jaakkola⁵, who also found higher levels among male Southern Finns in 1965–1966. Several other investigators, however, have found higher female levels^{1–4,6,7,11}. My measurement results can be explained by the sex difference in iron metabolism. If it is assumed that fallout ^{55}Fe was all released into the stratosphere in a single injection—say in 1962—human blood levels would start to increase after about 2 yr¹⁰. Because of their higher metabolic rate, female values would rise faster and reach a higher peak than the values for males. This would also cause a faster female decrease after peak time, and the clearance curves for males and females would cross after a certain time. This seems to have occurred in Norway before 1969. In accordance with this theory, the sex difference was found to be less pronounced in 1967 (Table 1).

Men and women between the ages of 18 and 76 participated in the study. They showed no statistical difference in values according to age, but a tendency towards lower values was observed during the twenties, and peak values occurred at about fifty years of age.

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Possible Significance of Filaments in Sieve Elements

OF the several theories of sieve tube translocation at present being investigated, the surface action theory of van den Honert¹ appears to have the least favour. Opponents to this theory have pointed out that the surface area requirements of such a system are beyond the capacity of the sieve tubes², but calculations based on evidence from electron micrographs make this a contentious issue.

Several workers^{3–6} have pictures of sieve plates of different species with the filamentous "p-protein" passing through the plates. Several authors have reported the diameter of these filaments to be within the range 60 Å to 240 Å. I have calculated the effect such filaments would have on the actual surface area within a single sieve element, making a number of assumptions.

First, the filaments have an even distribution through the pores and are continuous throughout the lumen⁷; second, 50% of the surface of the plate is "open" (a standard value for most calculations⁸); and third, 20% of the cross sectional area of the pore is occupied by these filaments.

With these assumptions 10% of the cross sectional area of the lumen would be occupied by the filaments. For convenience in calculation, a sieve element was treated as a right cylinder 250 μm long and 20 μm diameter, well within the range of sieve element sizes reported by Esau⁹.

In this instance, the cross sectional area of the sieve element would be $10^{-6}\pi \text{ cm}^2$, and the 10% occupied by the filaments $10^{-7}\pi \text{ cm}^2$. Taking a mean value of 120 Å as the diameter of these filaments, the cross sectional area of one filament would be $36\pi \times 10^{-14} \text{ cm}^2$. Thus the number of filaments, N , can be calculated for a single sieve tube as: $N = \frac{10^{-7}\pi \text{ cm}^2}{36\pi \times 10^{-14} \text{ cm}^2}$

i.e. $\frac{(\text{area occupied by filaments})}{(\text{area of one filament})} = 2.8 \times 10^5$ filaments in each sieve element.

Therefore, the effective surface area of the filaments in a single sieve tube would be $N\pi dl$ (where d is the diameter of the filaments and l is the distance between the two sieve plates) $= 2.8 \times 10^5 \times \pi \times 12 \times 10^{-7} \times 2.5 \times 10^{-2} \text{ cm}^2 = 2.65 \times 10^{-2} \text{ cm}^2$. The volume of a sieve tube of these dimensions would be $7.85 \times 10^{-8} \text{ cm}^3$, giving a surface area to volume ratio of $3.4 \times 10^5 \text{ cm}^2 \text{ ml}^{-1}$.

The generally accepted value⁸ of 10% (approximately 0.3 M) for the concentration of sucrose in sieve tubes permits a calculation of the number of sucrose molecules in an individual sieve element. In one sieve element of these dimensions there would be 1.41×10^{13} molecules of sucrose. The effective surface area within the sieve element was changed to equivalent planar dimensions, and the molecular capacity of the surface in terms of a monomolecular layer of sucrose molecules was calculated. Using a radius of 5.3 Å for the hydrated sucrose molecule¹⁰, the surface area available is equivalent to a monomolecular layer of 2.36×10^{12} molecules, within an order of magnitude of the number of sucrose molecules in the sieve element.

While it would be brash to assume that the entire population of sucrose molecules might be in association with the filaments, the calculations suggest these capacities may be approached. A surface phenomenon could explain bidirectional flow in individual sieve tubes^{11–13} and could account for minimal water movement^{14,15} if only the hydration spheres of sucrose molecules were moved. On this basis I believe that a physiologically mediated process on the surface of the filaments should not be discounted.

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BOOK REVIEWS

Life Elsewhere

Theory and Experiment in Exobiology. Edited by Alan W. Schwartz. Pp. 160. (Wolters-Noordhoff: The Netherlands, 1971.) Dfl.36.50.

At first sight it seems premature to announce the publication of a series of books on exobiology before there is any evidence that there is such a science—that is to say, that Earth is not the only site where structures worthy of the title living exist. The University of Nijmegen has, however, established a department of exobiology and the series will emanate from it; judging from this first volume, the subject will not be interpreted very strictly. Tentative themes to be treated in later volumes are listed, and from them it appears that pure speculation is to be excluded. Although speculation should obviously be kept within bounds, its exclusion may be a pity, for some of the authors in this volume assume that only one kind of life—our kind—is possible. They do not seem to realize that organisms do not adapt to a non-existent environment. Thus, the observed absence of terrestrial organisms able to multiply at temperatures above 100° tells us very little about the potentialities of organisms; it merely tells us, what we already know, that there are no extensive and long-enduring environments, containing substances capable of biologically useful interaction, hotter than that. A few centuries after such an environment has been maintained in conditions that permit the access of mutants, it will, if the environment remains sterile, be worthwhile speculating on the peculiar coincidence that the thermal upper limit of life equals the boiling point of water at terrestrial atmospheric pressure. Till then it is absurd to pontificate on the impossibility of any form of life in some environment because it is too hot or cold.

Interest in a series of books such as this will be focused on the extent to which articles presenting new information, or a new point of view, can be attracted. Three of the articles here are rather unsuccessful. One describes the present state of planning for flybys and landings, the kind of information that is hoped for, and the precautions to be taken against contamination. Another deals with catalysis by

substances other than conventional enzymes. It gives welcome space to azulmic acid and other cyanide complexes but deals mainly with the activities of "proteinoids" made by heating mixtures of amino-acids. The third points out that the solubility of calcium phosphate in pure water tells us little about the concentration of phosphate in the more complex solutions that can reasonably be postulated when water began to collect on the surface of probiotic Earth.

Sunlight is the dominant source of energy on Earth now. A great deal of attention has been given to the part it may have played initially in promoting organic syntheses and building up what Haldane called the primitive soup. Attention has also been given to electric discharges. Ponnampuruma and Sweeny discuss the possible role of radioactivity. Although they recognize that the average energy intensity of ionizing radiation was probably only one two-hundredth that of solar ultraviolet light, the energy is localized and deployed in veins of radioactive minerals. Anything synthesized at these sites could be protected from destruction so that the accumulation of organic matter would be facilitated.

All organisms use porphyrins; their presence in rock is therefore sometimes taken as evidence for the existence of organisms at the time when the rock was laid down. Hodgson disputes this conclusion and describes the non-biological synthesis of the porphyrin ring. He goes on to suggest interstellar synthesis and to consider the remote possibility that porphyrins are responsible for the colour of the great red spot on Jupiter.

Jupiter is dealt with in detail by Lewis and Prinn. Starting with the apparently agreed conclusion that Jupiter radiates more energy than it receives from the Sun, they argue that it has no solid core but is a convective system throughout, with a central temperature of 7,300 K. They go on to discuss how H_2 , H_2O , CH_4 , NH_3 , H_2S and so on would be expected to distribute themselves in shells on such a body and the syntheses that would be expected in these shells under the influence of solar ultraviolet light. Their candidate for jovial colouring matter is ammonium sulphide.

Only a small part of the surface of

the Moon has been explored, so it is still possible that large amounts of bitumen, which some had postulated, will be found. Kvenvolden surveys old speculations and summarizes the present analytical results. Analysts disagree in their conclusions, which suggests that some lunar samples are contaminated, but none find organic matter in more than trace quantities. The most interesting result is that, although the amount of carbon in lunar rock is similar to that in terrestrial basalt (150 p.p.m.), there is more ^{13}C than is usual on Earth or in the total carbon of meteorites. There is similar enrichment in a few terrestrial carbonates and the carbonate fraction of carbonaceous chondrites. These observations will probably ultimately shed light on the origin of the Moon but they have little immediate bearing on exobiology.

N. W. PIRIE

Making Connexions

Developmental Neurobiology. By Marcus Jacobson. (Developmental Biology Series.) Pp. xi+465. (Holt, Rinehart and Winston: New York and London, 1970.)

MARCUS JACOBSON works on the mechanisms by which neural connexions are specified and made. His book concerns the whole of the development of the vertebrate nervous system, with considerable excursions to other, more general, developmental topics. Of the 465 pages, 116 contain bibliography. Whatever its other merits the book is therefore a compendium; in my view it is essential to any biologist interested in the development, maintenance or regeneration of neural and neuromuscular connexions.

This having been said, I must also say that I find some of the early discussions in the book dogmatic. This is particularly true of those topics which are outside Dr Jacobson's special field of interest. This does not, for me, seriously detract from the book, but it will certainly irritate some readers. The later chapters, on the differentiation and growth of neurones and the ways in which they become connected, are well balanced, clear and interesting. In particular the coverage of early work is excellent and compre-

hensive, continually drawing attention to forgotten, but important, facts and techniques.

My greatest disappointment is that the discussion of theories of neuronal specificity is extremely short, and essentially devolves on one postulate, that interneurons are especially important in connexions that are formed late in development, or that depend on other than purely genetic information. However, this is a realistic assessment of the state of our knowledge and simply emphasizes its paucity. The book as a whole reveals the complexity of neural development, and clearly poses the problems to be solved. One of its best features is Dr Jacobson's insistence that much information can be built into a complex system by specification of the temporal order of events in its construction. The genetic load for this specification can be quite small, and, together with interactions with the environment, can lead to the development of a highly complex, partially plastic structure. In other words, repeated operation of a few simple rules can lead to exceedingly subtle and complex patterns of connexion and of behaviour. Indeed, it is becoming more and more apparent that an animal with a highly developed central nervous system, such as a cat or a man, depends critically on interactions with its environment for the development of many of the more complex functions, for example those involved in visual recognition. The problem of deciding how the genetic material can carry enough information in a vertebrate nervous system is seen not to be a problem at all; as in human language, the rules of the grammar are extremely complex, subtle and hard to discover, while the parts of speech are relatively simple.

ANTHONY ROBERTSON

Crystal Growth

Crystallization from Solutions. By E. V. Khamskii. Translated from the Russian by Albin Tybulewicz. Pp. viii+106. (Consultants Bureau: New York and London, 1969.)

INTEREST in all aspects of crystal growth has increased greatly in the past twenty years. The chief stimuli have been, on the fundamental side, appreciation of the part played by dislocations and other imperfections in the growth process and, on the practical side, the need for closer understanding and control of the growth of semi-conductor crystals. Growth from solutions, though it is practised on a very large scale in the manufacture of solid substances, has received less attention than growth from melts, pre-

sumably because the need for closely controlled physical properties is less urgent for substances crystallized in bulk from solutions than for those grown as individual crystals from melts. Nevertheless, many aspects of growth from solutions are of great interest, and indeed of great practical importance when the size, shape and purity of crystals in bulk products are involved. For this reason, Khamskii's book, which is a survey of the present state of knowledge of crystal growth from solutions, is a welcome contribution to a subject that has received less attention than it deserves.

Chapters on supersaturated solutions, nucleus formation and the growth of individual crystals are followed by a series on crystallization kinetics (induction periods and the rates of bulk crystallization), the coprecipitation of impurities, and the influence of impurities on crystal habit and on the physicochemical properties such as hygroscopy and caking. The accounts of these subjects are written in a clear, straightforward if undistinguished style and form a useful survey of the experimental work that has been done, especially the Russian work. The treatment of the theoretical attempts to understand the fundamental processes in molecular terms and to correlate different phenomena is less successful, and seems plodding and uninspired. For example, in the chapter on the growth of individual crystals, the style of exposition gives at first the impression that there are several rival theories—a diffusion theory, a thermodynamic theory, a molecular-kinetic theory of addition of particles and layers, and a dislocation theory. Eventually there is recognition that "The growth processes of real crystals are so complex that it is impossible to explain them using one theory". But this comes much too late; recognition right from the start that diffusion and the events at the crystal face are both important, that surface nucleation and the influence of dislocations must both be considered, and that thermodynamic and molecular-kinetic considerations are different approaches to the same thing, would be more appropriate and would provide the reader with a valid perspective for his thoughts. The task of theory is to give an understanding of the relative parts played by the various molecular processes in different circumstances and in different crystal species, and to relate the thermodynamic and molecular approaches.

Another example of rather uninspired treatment is in the chapter on the influence of impurities on crystal habit. Selective adsorption of an impurity on certain crystal faces, the part played by the relative solubilities of the two substances, the relation between the struc-

tures of the impurity and the substrate, and the possible relevance of the existence of double salts, are all mentioned, but no attempt is made to correlate these things, and the reader is left with the impression of a welter of observations and ideas with no connecting thread. An integrated treatment of selective surface adsorption, epitaxy, and mixed crystal and double compound formation in terms of crystal structure would illuminate this section and again provide a valid perspective for the whole subject.

These criticisms of the theoretical treatments should not be allowed to weigh too heavily; the value of the book is in the large amount of factual material in a moderate compass, and the general survey of the whole subject supported by a comprehensive list of references. It would be a useful addition to the library of anyone working in this field, especially because it summarizes a great deal of Russian work which may not be familiar to English-speaking readers.

C. W. BUNN

Hot Plasmas

Physics of Hot Plasmas. Edited by B. J. Rye and J. C. Taylor. (Scottish Universities' Summer School, 1968.) Pp. xv+455. (Plenum: New York; Oliver and Boyd: Edinburgh, 1970.) £14.95.

ONE would not claim that this book makes easy reading (especially because of the paucity of cross-referencing from one author to another); but, except for that (surely very small) minority that can keep up with the vast plasma literature, everyone with some professional interest in the field is sure to find much to catch his attention.

Space permits only a somewhat invidious selection of chapters for discussion. W. B. Thompson begins with the Liouville equation and the B-B-G-K-Y hierarchy of distribution functions for interacting particles, with the inevitable truncation problem. The Boltzmann and relaxation approximations are developed and their limitations pointed out—for example the violation by the relaxation treatment of the Onsager relations, and the necessity for a plausible but still somewhat *ad hoc* cut-off to the collision integral at small angular deflexions. A model more appropriate to the long-range nature of the Coulomb interaction is developed, but—presumably because of the use of the random phase approximation—it is by contrast unable to deal with the *strong* interaction end and appeal must be made to the Boltzmann equation for the large-angle cut-off.

E. G. Harris begins by noting that a recent review listed no less than 31 different instabilities; but this apparent increase in theoretical chaos has been

accompanied by a better understanding of the common features within families of instabilities. For example, a number of modes near the ion cyclotron frequency and its harmonics—discovered at different times and bearing a variety of names—can all be looked on as depending on an “inverted population”, as in a maser, with consequent emission of plasma waves by particles exceeding their absorption. The large number of instabilities is due to the distribution function's dependence on six variables rather than just the energy, so that there are many more ways of inverting the population.

The limitations of linear instability theory are emphasized early on by M.G. Rusbridge, who remarks that whereas almost every confined plasma is theoretically unstable to rapid exponential growth of a wide spectrum of modes, in practice most experiments show a quasi-stationary spectrum of random fluctuations. This clearly implies that non-linear effects have altered the plasma sufficiently so as to suppress the predicted growth; but the quasi-linear approximation cannot predict the correct fluctuation level at which amplification is halted. There is some superficial similarity to ordinary hydrodynamic turbulence, with non-linear coupling between modes limiting growth, and stochastic acceleration of particles by the random electric fields of the waves (“Landau damping looked at from the point of view of the particles”) the analogue of viscous dissipation. As long as the turbulence is weak the fluctuations still consist almost entirely of resonant modes, obeying closely the linear dispersion relation; but the non-linear coupling generates a weak field of non-resonant modes—“virtual waves”—which, however, are real enough to suffer Landau damping and to form a link in four-wave interactions. Equations have been derived for the variation of the turbulence spectrum, with the truncation problem bypassed by the random phase approximation. Rusbridge is sceptical of the steady-state solutions in the literature, however; he adduces experimental support for the possibility that the level of turbulence must inevitably rise until the characteristics of weak turbulence—in particular, the clear distinction between resonant and non-resonant modes—disappear, so that only *strong* turbulence can be statistically steady. Because of his distrust of the basic theory he remains unconvinced by current explanations of anomalous transport phenomena in terms of plasma turbulence.

C. Oberman's chapter (drawn largely from the Princeton PhD thesis by A. Rogister) is the most exacting in its mathematical complication. The plasma is described by non-linear Vlasov equa-

tions, but with singular rather than smooth initial conditions, so as to reflect the discreteness of the plasma particles: “discreteness effects and collective behaviour are not separable.” The author is confident that the formalism adequately covers not only weak turbulence but also the regimes of strong turbulence dominated by wave absorption and emission. One looks forward to a detailed comparison with observation of the predictions of this highly ambitious theory.

Enough has been said to indicate that this book will make a valuable addition to the plasma physicist's library; one feels that there is much less here of ephemeral interest than in the published proceedings of most summer schools.

L. MESTEL

Basic Geomorphology

Introduction to Geomorphology. By Alistair F. Pitty. Pp. xvi + 526. (Methuen: London, October 1971.) £5 cloth; £2.50 university paperback edition.

GEOMORPHOLOGY of a scientific kind is about one hundred years old, the first perceptive text being that of Oscar Peschel entitled *Neue Probleme der ... Morphologie der Erdoberfläche* in 1869. But the broad basis of the subject had been laid a century earlier by hydraulic engineers and geologists, whose findings were derived from laboratory experiments and field observations. Unfortunately civil engineers and geologists or physical geographers often lost contact with each other and landform studies became dominated by concepts of shape and of sequences of erosional stages rather than of the processes involved. Almost inevitably in recent decades increasing emphasis has been put on the dynamics of processes based on laboratory and field measurements and expressed in mathematical terms.

Dr Pitty's book attempts to do justice to this kind of work done since 1945 but “strives to avoid a too uncritical acceptance of contemporary trends”. He devotes the first twenty per cent of his total informative text to an examination of the fundamental characteristics and basal postulates of geomorphology. In this he draws a number of conclusions but does not evolve any canons or rules or laws, and does not evaluate any systems approach in the way that Chorley and Kennedy have done in their *Physical Geography* (1971). This may well be because “few landforms, if any, are understood with certainty”.

The following chapters attempt to define the systematic stages through which the study and interpretation of the landforms of a given area might proceed. Thus the main part of the

volume deals with methods rather than with the presentation of landforms topic by topic. The emphasis is laid squarely on the physical, chemical and biological bases of geomorphological processes and on the inter-relationships between these processes and observed landforms. These aspects are demonstrated by details of actual observations, with ample statistical and diagrammatic support.

There follow a section on “stages” in the evolution of slopes, drainage systems, glaciated areas and coastlines; an appendix on some simple methods of field measurement, and an excellent “selected bibliography” of 74 pages. The student can also be grateful for a modest price.

In an adventurous and progressive guide-book on a contentious subject it is easy to criticize the author's choice of emphasis and content. Dr Pitty is primarily concerned with microforms and almost considers sub-crustal movements and mountain-building “as irrelevant and inappropriate to geomorphology”. He thereby obscures the first principle of that science: “Our land has two extremities; the tops of the mountains, and the sea-shores: where there is a seashore and a higher ground, there is that which is required for the system of the world” (James Hutton, 1788). Continental drift is also dismissed summarily with unfortunate results. We are told of the permanency of the ocean basins and confronted with a fine diagram showing that the Atlantic Ocean did not exist a few hundred million years ago. Elsewhere the text avers that in the Eocene a deep weathering crust developed in Ireland at 55° N, so presumably that island avoided the sub-crustal conveyor belt.

It must be stated, however, that no existing single volume introductory text deals so well with so many microforms. This aspect alone will make the volume attractive to all students who like field-work. The examples have a wide geographical distribution and range intellectually from von Kármán's constant to mole hill formation. They may at times seem trivial, as does the full page devoted to new tree sprouts as a guide to floods on the Potomac, 1928–1955, but their effect will be to arouse enthusiasm for field observations. New editions may be confidently expected and will give the opportunity to improve some definitions and terms; for example, the Geikie brothers need not be insulted; Davis needs re-assessing in the light of a large volume in the press; Monterey is not in Mexico; and it seems incredible that “the factors of aspect and exposure in landform studies cannot be underestimated”.

ROBERT P. BECKINSALE

Explaining Disease

A History of Medicine. Edited by Lester S. King. Pp. 307. (Penguin: Harmondsworth, 1971.) 60p.

THE compiler of this work is an authority on the history of medicine, and is also the editor of the *Journal of the American Medical Association*. Among his previous writings are a general survey of medical thought, several substantial papers and two wise studies of eighteenth century medicine. It follows that he is excellently qualified to edit an anthology such as the one under review.

In it he presents extracts from some of the works that, in his view, make rational attempts to explain the mysteries of disease. Each passage, of which there are twenty spread over 265 pages, is preceded by a short introduction. The whole work is prefaced by a condensed but useful account of medical history in which Dr King also expounds the principles that have guided him in making his selection. A subtitle for his book, he suggests, would be "a study of critical acumen".

There are four main sections. The first, headed "Classical Heritage", covers Hippocrates and Galen; the second, "Revolt", includes Vesalius, Paracelsus and Harvey; the third, "Development", takes in Sydenham, Boerhaave, Cullen, and so on; and the fourth, "Fruition", brings us through Virchow, Bernard, Banting and Best to a recent article in the *Journal of the American Medical Association* by Simon and Harley.

As with any anthology many names are left out and many questions left begging. There is no Pasteur, for instance: no Snow, no Semmelweis (true, he has never been translated), no Laënnec, and absolutely no Arabs; but on that score we have no real criticism to offer, for the space is simply not available to accommodate them all. What we would question is the editor's choice of Hahnemann, despite the admission that "homeopathy was a rather curious medical backwater" and despite the total exclusion of mediaeval medicine on grounds that are not so very different ("it did not contribute much to the critical acumen whose development forms the pattern of this book"). In the context of medical history one would have a hard time defining what "critical acumen" really is. If it is to be found in Hahnemann why not also in the theories of the miasmatisers, or indeed of the eighteenth century Brunonians who, lacking the technical facilities that we take so much for granted today, were driven to weaving brilliant conceptual patterns and to making apparently logical, but no less mistaken, deductions.

But whatever slight reservations one has to make about Dr King's choice,

on the whole his anthology must be welcomed as a solid and reliable piece of work. Perhaps it is a little unadventurous and conventional, but, that being said, it has the very definite ring of authority. We can be certain that considering the ubiquity of Penguins it will reach the homes of many who as yet hardly suspect the existence and fascination of medical history, and this surely will be a good thing. It may even entice some readers to reach out for the complete versions of Dr King's abbreviated texts.

E. GASKELL

Women at Yale

Women at Yale. By Janet Lever and Pepper Schwartz. Pp. 274. (Allen Lane: The Penguin Press.) £2.50.

THERE is, or used to be, a Harvard song about the unhealthy effect the lack of females had on students at Yale University. If this book, describing the experience of Yale's first year of co-education, is accurate, the song will not have to be abandoned. There still exists, it seems, a hell of a situation down at Yale.

The presence of 500 women among 4,500 undergraduates seems to have thrown both sexes into a state of sexual and intellectual shock. That was not the intention behind co-education. Yale, as the world knows, is an enlightened place, run by Kingman Brewster who is the shining example of a college president who knows when to give in and when to stand firm. He recognized that the hour of women's equality had come and yielded to the undergraduate demand for women on campus, even though it meant offending the old graduates on whom much of Yale's financial support depends. In the autumn of 1969 women entered Yale and went to live in its residential colleges where the male students also live. Yale's plan, which is proceeding, was to have their numbers increase until both sexes are equally represented among the students.

But co-education proved more uncomfortable in practice than in theory. The men began to realize that they would be deprived of the "Yale experience" which nurtured their fathers and grandfathers and Dinky Stover before them. Theirs was not to be four years of cloistered fraternity with the future leaders of the East Coast establishment. Instead the elite was to be diluted by the presence of outsiders who, in addition to being female, offered little promise of distinction in later life. And what might have been a compensatory advantage—the availability of feminine companionship during the Monday-to-Friday period during which Yalies have traditionally

gone without—turned out to be a liability. The men soon realized the awkwardness of having to confront at breakfast the bedpartner of the night before. The solution to this widely experienced dilemma was what the authors call "the incest taboo". Men and women from the same college found it simpler to remain just good friends.

As for the girls, they too found Yale disillusioning. A bright lot, good at their books, they found themselves stared at as sexual objects. Presumably this had happened to them before but the authors assert that at Yale it was worse, that Yale is a caricature of modern American male chauvinist society. The women therefore found themselves losing self-esteem. With eight men to every girl, the female who did not have a date on Saturday night knew that she must be repulsive beyond a doubt. And the Yale girls became hostile to the dates that Yale men imported, as is their tradition, at weekends.

This book makes good light reading and for that reason constitutes a disservice to the cause in which the authors, two women graduate students in sociology, fervently believe—women's liberation. They are obsessed with the idea of a university as a processor of people, an instrument of socialization. They mention hardly a word about academic work or intellectual activity of any sort. They also concoct a kind of mock sociologese that would do better in a teenage advice column than in what they insist is a serious book. They worry about the "friend-date dichotomy", exclaim over the "dangers of ego-protection" and congratulate themselves on their unique role as "both teacher and date".

One can only hope that their utter absorption in the dating ritual and lack of interest in the academic side of Yale is idiosyncratic and not general. Did nobody read a book that year? Were there no women arrivals who found the intellectual resources of a great university some compensation for the discomforts of being a sexual pioneer? It is possible that the presence of women did just what the male chauvinists have claimed for centuries—drove out thought. Or, possibly, the authors themselves are guilty of using the scene to make their point—that Yale, like the United States, has a social structure repressive to women. Perhaps the answer to the question of whether Yale has been liberated or not should not be attempted for another few years, to give time for the first women students to assess their relationship to the university itself and not just to the apprehensive young men who made their first year so self-conscious.

BRENDA MADDOX

CORRESPONDENCE

The Duke and ZPG

SIR,—You are quite right to stress that the rate of population growth is influenced by death rate as well as birth rate; and as you imply, people will not be persuaded to accept a decreased life expectancy. What you fail to appreciate is that this means that the only way of halting a still-increasing population is to reduce the birth rate sufficiently to compensate for the lower death rate. Such a policy would eventually result in a decrease in population, assuming constant fertility.

You are also ignoring the special problems of a nation like Britain which relies for its survival on imports. As world resources are depleted, and demand rises owing to increased population and industrialization, the pressures to become self-sufficient will grow. From this viewpoint, it is certainly arguable that Britain is overpopulated. As you suggest, the superficial evidence of crowded towns is not enough to go on. One needs a criterion for deciding if a nation is overpopulated, and we suggest that if a country's material and agricultural resources are insufficient to give it a reasonable degree of independence then its population is too large. By this criterion, of course, Britain has far too many people.

Next, we should like to point out the mistake of isolating population as a factor affecting the quality of life. Rather one ought to consider that a small increase in a highly industrialized population has a much greater impact on the environment, in terms of both pollution and demand on natural resources, than a corresponding increase in a non-industrialized nation. Nor should one overlook the folly of failing to practise what one preaches. It is easy for undeveloped nations to misinterpret the motives of someone who urges "one law for the rich and another for the poor".

Finally, we should like to emphasize the distinction which exists between "family planning" programmes designed to allow any woman the freedom to choose the size of her family and effective population control.

Yours faithfully,

C. J. BOLTON

J. E. CORDWELL

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Institute of Hydrology

SIR,—The italicized postscript to the feature article "Lord Rothschild and the

Institute of Hydrology" (*Nature*, **235**, 8; 1972) states:

"The Rothschild report also recommends that the Nature Conservancy, now part of the Natural Environment Research Council (NERC), should be transferred as a whole to the Department of the Environment."

In fact, the Rothschild report made no such recommendation. What is recommended was:

"The whole of the Countryside programme should be financed by the DOE and SDD, which explains why the Nature Conservancy (excluding basic ecological research (£0.25 m)) should be paid for by these Departments."

As the article itself correctly states, only the Institute of Hydrology among all the institutes of all the Research Councils is recommended for physical transfer, together with its staff, to a Department. It is the purpose of the article to describe the true nature and aims of the work in progress at the Institute, which consists largely of basic hydrological research.

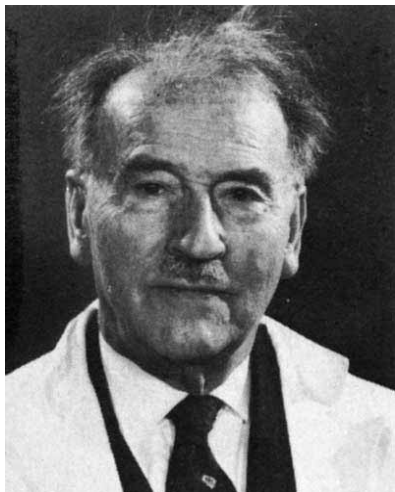
Yours faithfully,

R. J. H. BEVERTON

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Obituary

Professor Antoine Lacassagne



Courtesy of Fondation Curie

PROFESSOR LACASSAGNE, French pioneer in radiology and cancer research, died in Paris on December 16, 1971.

Antoine Lacassagne was born at Vil-

lerest (Loire) on August 29, 1884. He came of a medical family, his father having been a military physician and Professor of Legal Medicine in the Faculty of Medicine and Pharmacy of the University of Lyon. Doctor of medicine and intern of the hospitals of Lyon, in 1908 Professor Lacassagne entered the Laboratory of Histology to work under the direction of Claudius Regaud. He made the subject of his first scientific work "Histological and Physiological Studies of the Effects Produced on the Ovary by X-irradiation". In 1909 Dr Regaud was recalled to Paris by Emile Roux, Director of the Institut Pasteur, to take charge of the recently constructed Laboratory of Radiophysiology, Pasteur Laboratory of the Institut du Radium. In 1913 Regaud in turn sent for his pupil, Lacassagne, to install him in the new venture. Mobilized in 1914 Lacassagne then spent four years as medical auxiliary at the front, notably in Greece and Roumania. He returned to the Institut du Radium in 1919 where he served

to the end of his life. He was nominated Assistant Director of the Pasteur Laboratory in 1923. In 1937 Regaud retired and Lacassagne succeeded him as Chef de Service of the Institut Pasteur and Director of the Section of Biology of the Institut du Radium. At the same time he became Director of Research in the Fondation Curie which had been founded in 1921 as a department for the practical application of the work of the Institut du Radium. These positions he held until his retirement in October 1954. In 1941 he was elected Professor of Radiobiology and Cancerology in the Collège de France. Also in that year came his election to the Académie des Sciences. Retirement did not diminish his activity, and he retained a laboratory in the Institut du Radium where R. Latarjet had succeeded him. In 1957, on the death of Justin Godard, he became President of the Ligue Nationale Française contre le Cancer and remained in this post till the time of his death. In 1950 Lacassagne acted as President of the Fifth

International Congress held at the Sorbonne under the auspices of the Union Internationale contre le Cancer, the first of the series to be held in France. He was a member of many foreign academies and honorary doctor of numerous universities. He was Commander of the Légion d'Honneur and Grand Officer of the National Order of Merit.

Professor Lacassagne was primarily a pathologist, but his work ranged widely to include experimental radiopathology and endocrine carcinogenesis. He was also a pioneer of the new and developing subject of radiobiology. From the close of the First World War he collaborated actively with Regaud in that fundamental work which rapidly led to a scheme of treatment of cancer in man, by X-rays, radium and radioactive isotopes, and was foremost in the development of autoradiography. With the physicist, Fernand Holweck, he continued the study of the fundamentals of radiology, especially through the use of microorganisms, and pursued this work further with Wollman. From 1929 with R. Vincent and others, Lacassagne made a study of the carcinogenic response of infected foci following irradiation. In 1932 came the discovery of the role of oestrogens in causing cancer of the breast in male mice, an observation which greatly stimulated endocrine carcinogenesis and was the forerunner of the successful treatment of carcinoma of the prostate with oestrogens.

From 1943 he took a leading part, inspired by the work of Otto Schmidt, in attempts to relate the biological activity of the carcinogenic hydrocarbons to the molecular distribution of π electrons with the collaboration of P. and R. Daudel, A. and B. Pullman, N. G. Buu-Hoi, G. Rudali and F. Zajdela.

Professor Lacassagne was a true savant and humanitarian, whose worldwide influence will be greatly missed.

Professor Aleksandr G. Vologdin

ALEKSANDR GRIGOREVICH VOLOGDIN, one of Russia's leading palaeontologists and geologists, died on September 28, 1971, aged 75, after a long illness.

Vologdin was born on March 11, 1896. He was educated at the Leningrad Institute of Mining, from which he graduated in 1925. His activity in geology began, however, prior to his graduation—from 1920 onwards, he served on the Geological Committee of the USSR, and remained associated with this and subsidiary institutions until 1943.

He became a specialist in the geology and stratigraphy of Siberia and the Asian regions of the USSR. At first his main concern was prospecting for mineral resources, but this work led to an ever-growing interest in the palaeontology of the region, particularly that of the Cambrian strata.

From 1931 to 1966, he published a number of definitive papers and monographs on the archaeocyaths found in these areas. These works include: *Archaeocyaths of Siberia* (1931), *Archaeocyaths and Water-Plants of the Cambrian Limestones of Mongolia and Tuva*, *New Data on the Archaeocyaths of Siberia* (1959), *Three Types of Archaeocyaths from the Siberian Cyath Lower Cambrian Zone of the Baikal Up-lands* (1959), *Stromatoliths and Phototropism* (1963), *Discovery of Triple-axial Symmetry in Cambrian Archaeocyaths* (1966).

He also published detailed geological surveys of the Tubinskii-Sisim and Kizir-Kazyrskkii Regions and of Northern Bukovina and Bessarabia.

In 1939, he became a corresponding member of the Academy of Sciences of the USSR, and in 1943, an associate of the Institute of Palaeontology of the Academy. He worked in association with the leading Soviet geological and

mineralogical bodies, including the All-Union Aero-geological Trust, the Institute of the Petroleum and Gas Industries, Moscow State University and the Soviet Ministry of Geology.

Announcements

Appointments

Sir Alastair Pilkington has been appointed to the Science Research Council in succession to Mr D. L. Nicolson.

Professor C. A. Coulson has been made president of the Institute of Mathematics and its Applications. The new vice-president of the Institute is Professor Heini Halberstam.

Miscellaneous

The CIBA medal and prize of the Biochemical Society has been awarded to Dr D. H. Northcote, University of Cambridge, for his structural and biochemical studies on the formation of the cell walls of plants. The Colworth medal of the Society has been won by Dr Alan R. Williamson, National Institute for Medical Research, Mill Hill, for his work on gamma-globulin synthesis.

The Society of Pharmacological and Environmental Pathologists was founded in October 1971 to foster the discipline of pharmacological and environmental pathology, to contribute to the development of criteria and standards for the evaluation of pathological changes induced by drugs, chemicals or environmental pathologists, and to establish a registry of naturally occurring and induced diseases in laboratory animals. The president of the new society is Dr C. Hans Keysser. Those interested in the society are invited to write to the secretary-treasurer, Dr Howard A. Hartman, PO Box 276, Florham Park, New Jersey 07932, USA, for further information and forms of application for membership.

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